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Intestinal tract..

# The Intestinal Tract

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## STRUCTURE, FUNCTION AND PATHOLOGY IN TERMS OF THE BASIC SCIENCES

*By*

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*To my family, for understanding*

# PREFACE

THE NEED for a reference volume on the intestinal tract becomes apparent when an attempt is made to obtain a description of its activities from scattered literature references or general text books. The present work is designed to fill part of this need by presenting a source of data basic to an understanding of the intestine. A discussion is given of intestinal structure, function, and pathology so that physicians, research workers, and students can have at their fingertips data which have been quite scattered. Intestinal activities are presented, when possible, in terms of measurable parameters. Methods developed in the last decade supplement older procedures and permit an evaluation of alimentary function with some accuracy (techniques such as mucosal biopsy during life, analysis of enzymes translocated from the intestine to the blood stream, and the use of radioisotopes to follow digestion, absorption, and enzyme production).

Intestinal performance is presented from the view point of the basic processes involved. Ideally, the prime focus of such a study is the individual cell. However, until data concerning the metabolism of each type of intestinal cell are available, we can cautiously rely on fragmentary reports in interpreting normal variations and the altered processes of disease states. Studies relating to the subject, originating from both clinical and laboratory groups, are located in diverse journals. The first task has been compilation, for the availability of this information is prerequisite to its use. The second task has been exposition and correlation, so that a somewhat comprehensive picture emerges. Much of our information has been gathered during studies of disease processes, and attention is given, particularly in the second half of the book, to clinical alterations.

This however is not primarily a book about "bedside gastroenterology" in the usual sense (several such volumes are available and provide dosage schedules and other clinical details). Rather, this is a presentation of data necessary for the interpretation of intestinal function and dysfunction. In areas in which clinical aberrations are apparent but no basic data are gathered, findings have been presented, of necessity, in clinical terms. The book may thus serve as a reference for clinical studies, an introduction to the pertinent literature for scientists, and a text for students. No pretext is made to exhausting the subject or including all pertinent references. In a number of instances, more recent but less definitive papers have been listed because of their contained bibliographic material; hence the listing of an article does not necessarily mean that it has chronologic priority. My debt to those who have contributed reviews of the literature is obvious. Certain subjects of great interest in themselves, which have been extensively dealt with elsewhere, are only mentioned in passing. Thus the areas of intraluminal pressure changes, theory of active transport, the mucous barrier, intestinal motility and the portal circuit, are discussed briefly and further reading sources suggested.

During the digestive process, secretions from the stomach, liver, pancreas, and intestine are churned together with food materials. Digestion and absorption take place from this complex medium which is a clinical reality, but a biochemist's nightmare. It is impossible to analyze the situation in a few words or a handful of equations. Out of necessity, the subject has been subdivided for ease of presentation. It should be borne in mind that the body recognizes no such divisions. Acts within the intestine take place sequentially, usually under excellent coordination. We lack information as to the overall mechanisms integrating such acts; this is but one of the problems whose solution awaits the future.

Errors of omission and commission are all too likely to appear in such a volume. For these the author expresses regret, and hopes the reader will call them to his attention. The most serious lack though, is a paucity of data to illuminate, correlate and define the varied aspects of intestinal function. Information will have to be gathered from both the clinic and the laboratory to fill in these blanks.

# ACKNOWLEDGMENTS

Several physicians and scientists gave of their time to read sections of the manuscript and provide valuable comments. Dr. Norman G. Levinsky of the Massachusetts Memorial Hospitals reviewed extensive portions of the book and helped clarify a number of points in the comparison between the kidneys and the intestine. Certain other chapters and sections were read by Dr. Norman Zamcheck of the Boston City Hospital, Dr. Richard F. Shaw of the University of Virginia School of Medicine, Dr. Bernard J. Haverback of the University of Southern California School of Medicine, and Drs. T. Hastings Wilson and Manfred Karnovsky of the Harvard Medical School. To these reviewers I am deeply grateful. My thanks are also due to those who allowed copyrighted material to be reproduced, to the Navy Medical Department and Miss Marilyn Costello for the illustrations, and to Mrs. Diane Riley for producing a typewritten manuscript from scattered notes.

R. P. S.

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# The Intestinal Tract

# I

# THE INTESTINAL CANAL AND METHODS FOR ITS STUDY

# 1

# DEVELOPMENT, GENETICS, AND STRUCTURE

WHILE THE anatomic picture of development is being drawn in the embryo, there also occurs, more fundamentally, an emergence of the biochemical characteristics of the individual. It is probably not incorrect to say that this pattern decides the pathways along which the organism will function. "Normal variations" appear in the process, and are reflected in minor differences which help distinguish one being from another. The intestinal tract, for example, shows variations in weight,[22] length,[75] intraluminal amino acid composition,[1,2] enzyme content[96] and other parameters. Knowledge of the mechanisms responsible for such changes is still fragmentary.[3,4] As differentiation proceeds, there is a loss of synthesizing abilities;[5] a number of enzymes widely distributed in embryonic tissues become preferentially concentrated. Alkaline phosphatase[6] and other ferments can be demonstrated in the embryonic duodenum, but no clear view has yet evolved as to the enzyme pattern or functional responses of the fetal intestinal tract.[7]

Mechanical activity of the developing gut begins at an early date. Amniotic fluid is swallowed by the fetus as early as the fifth month,[8] and some defecatory-like movements are present.[9] Following the introduction of thorotrast (thorium dioxide, a radiopaque material) into the amniotic cavity 24 hours before delivery, the material can be demonstrated in the fetal pulmonary tree, as well as the esophagus, stomach, and entire intestinal tract.[10]

Air is swallowed with the first extrauterine breath or shortly thereafter. Hence inability to demonstrate gas within the intestine, a day after birth, is presumptive evidence of intestinal obstruction. In addition, distention caused by air is probably a stimulus to the initiation of the digestive process. Bacteria enter with swallowed air and become a permanent part of intraluminal events.

The absorptive surface of the intestine of the newborn is apparently adequate, secreting glands are functional, and the gut itself is thin and readily distended.[11] However, the intestine continues to 'mature' after birth. This is indicated by several observations.

1. Following birth, there is a steadily decreasing ability to absorb antibodies form the gut lumen.[12,103] In some species the uptake of intact antibodies completely disappears after a few weeks of age. It is interesting to note that colostrum contains an antitrypsin which may perhaps act to prevent the digestion of its antibodies, so that immune globulins may be absorbed intact.

2. While not initially present, several days after birth, a system develops for the active absorption of selected carbohydrates in the chick.[13] The uptake of some sugars is then favored over that of others. Certain enzymes from digestive glands emptying into the intestine also increase in activity as the animal matures.[97]

3. An intermittent sucrosuria in mentally retarded human infants (who also have hiatal hernia) has been tentatively linked to the maturation of an immature intestinal enzyme system.[14]

Digestive enzymes are present in the gut lumen of the newborn, and the baby can soon handle 16 ounces of milk per day (about one-seventh of its birth weight). There may be an inability to cope with large amounts of carbohydrate in the diet (as evidenced by diarrhea) during the first year of life. Protein digestion is often adequate but claims have been put forth that the absorption of incompletely split proteins may result in allergy. Total transit time of ingested materials from mouth to anus can be less than proportionally shorter than in adults; the adequate gastrocolic reflex of infants soon makes itself known to all parents.

Usually between the first and fourth postnatal days, meconium is cleared from the alimentary canal. Meconium is a thick mixture of secretions, bile pigments, desquamated and swallowed cells and fluid, vernix and calcium soaps. Normal meconium contains but little protein. The tenacious substance in meconium ileus (accompanying cystic fibrosis of the pancreas) contains considerable protein, principally serum albumin.[15] Both meconium plugs and fecal plugs have been reported in the newborn,[16,17] in addition to intraluminal calcifications.[18] Moist, green 'transitional stools' can occur after the passage of meconium until normal infant defecations supervene about the first week.

## INTESTINAL TRACT OF OTHER SPECIES

Comparative aspects of intestinal development and function are usually not stressed, despite their importance in enhancing our understanding of the human gut. The ruminant intestine has recently received some attention. Benzie and Phillipson[19] presented illustrations of this type of digestive canal, and reviews[20,88] have summarized the metabolism of the ruminant and the role played by bacteria during the intraluminal breakdown of ingested grasses. The intestinal tract of marine organisms has a part in altering clay minerals,[21] and the passage of soil through the gut of the earth worm is an important factor in keeping the ground porous. An interesting variant of intestinal function, with vast economic consequences, is found in the larvae of the clothes moth. Within the digestive system of this creature is a high concentration of a compound (s) which reduces the disulfide groups of wool to sulfhydryl radicals (-S-S- ⟶ -SH), producing an end product that is readily attacked by proteolytic materials in the intestinal tract.[91] The otherwise unassailable wool is thus made into suitable food for the moth.

The recovery of a number of supposedly extinct Coelacanthidae from the deep waters off Africa has permitted careful examination of these 'living fossils'.[92] The anatomic plan of the gastrointestinal canal is similar to that of present species, indicating that the basic design was evolved early in the development of modern day forms. Interestingly, porphyrins (which are a normal component of feces)[98] have also been identified in the fossilized excrements of certain species.[93]

## EMBRYOLOGY

The human digestive tract is formed from two sources—an entodermal tube and an encasing layer of splanchnic mesoderm.[23] Epithelial cells of the intestine and its glands evolve from entoderm, while the mesoderm yields connective tissue, muscle, and peritoneum. The cephalic portion of the future digestive apparatus begins an earlier differentiation than more caudal regions. The reasons for this are not clear, and may involve an interplay between genetic factors, positioning of cells, and perhaps even activity of the primitive nervous system (in the insect Leucophaea, severance of the autonomic recurrent nerve results in the development of tumors in organs innervated by the nerve, particularly the anterior portion of the gastrointestinal canal [24]). Human embryos, and those of other mammals, show a gradient of certain activities (physiological and biochemical) with the most active region at the cephalic end. Theories concerning this polarity are discussed in the section on Metabolic Gradients.

During the early period of development, the mesodermal wall is not static, but starts laying down muscle fibers. Intestinal circular muscle appears by week seven, and longitudinal muscle is evident by the twelfth week. Linear thickenings of the longitudinal bundles form the three muscular tenia of the future large bowel. During the eighth week of development, while the human fetal intestine has circular muscle but no longitudinal muscle or intramural nerves, contraction rings may pass in either direction (orally or aborally [25]). The intestinal muscles persist in transplanted embryonic rat gut,[26] and such preparations may be used for the detailed study of developing smooth muscle. Carey proposed a theory that the intestinal muscles were arranged in the form of a spiral tube, and presented a discussion of its origin.[27]

In the embryo at four weeks, a simple tube stretches from stomach to cloaca. This primitive intestine is joined to the yolk stalk, and hence is divided into cranial and caudal loops which are supported by a dorsal mesentery. By the fifth week, the yolk stalk attachment has disappeared. It is then possible to identify, in the caudal intestinal loop, a bulge that is the developing cecum; hence there is delineation of the small and large bowel. Closely following these actions, an anticlockwise rotation of the intestinal loop occurs, with the superior mesenteric artery as the axis of rotation.[29,30] Accelerated growth throughout the length of the tract continues, and the gut is thrown into a series of coils. At about week seven, the abdominal cavity can apparently no longer accommodate the increased bulk, and the intestinal loop herniates into the umbilical cord.[31] Three weeks later, the abdominal space has once again attained sufficient size to hold the gut, and it returns to its former locale rather rapidly.[32] The small intestine enters first, and when the entire gut has returned, the cord coelom is obliterated behind.[23,30] The large intestine is the last segment to leave the umbilical cord, but when it does, the cecum becomes fixed in its position near the right ilium.[33]

The laggard of the developmental process, the ascending colon, starts its elongation in the midfetal period, and its growth is not completed until childhood. The distal portion of the cecum bulges to form a blind sac—the characteristic and famed vermiform process (appendix). At week 10 in utero, a bend separates the cecum from the colon proper, but in some adults the valve between cecum and colon may not be distinct. Mesenteric attachments of the ascending and descending colon are lost, as the loops are pressed against the body walls.

Subdivision of the cloaca, the 'common vent' at the caudal end of the entodermal tube, forms the rectum during the seventh week. Near the end of the eighth week, the cloacal membrane ruptures. Rectal entoderm is then in direct continuity with ectoderm from an infolded pouch of skin (the proctodeum); hence the anal canal is formed.[34]

The epithelial lining of the duodenum proliferates, and leads to luminal occlusion during weeks six to seven. However, vacuolation eventually restores the patency of the lumen. This process is shared, to a lesser extent, by the entire small and large intestine. Initially narrower than the small bowel, the large gut increases in diameter prior to the fifth month of development. Also present by the fifth month are lymph nodes and Peyer's patches. Other structures appear in sequence, with villi developing at the eighth week from rounded elevations in the epithelium[35] (even making a transient appearance in the fetal colon). At the base of the villi, during the third month, ingrowths of tubular epithelium form the intestinal glands of Lieberkuhn. In the duodenum, the compound glands of Brunner soon follow. Dynamic and progressive, the developmental process continues during childhood, with glands and villi probably increasing in number. Other aspects of enzyme development are discussed in the chapter on Production and Translocation of Gastrointestinal Enzymes.

## DEVELOPMENTAL ANOMALIES

The causative factors in congenital anomalies are incompletely understood. Poor maternal diet, anoxia, other trauma, infectious agents, and genetic factors may all play a part. The developing fetus is most sensitive to X-irradiation at about weeks 2.5 to 6, and Rugh[28] has discussed some of the effects on the gastrointestinal tract and other systems. In terms of the developmental sequence, the more common errors can be readily interpreted (see also Bremer[36]).

### 1. Meckel's diverticulum

Failure of the proximal yolk stalk to detach from the intestine (at the 9 mm stage), results in an ileal diverticulum. This anomaly is found in 2 per cent of the adult population. Various gradations of detachment of the yolk stalk occur,[37] forming cysts or umbilical fistulae. Other true diverticuli can develop at any point in the alimentary canal, including its upper reaches and the colon.[79] 'False diverticuli' occur as an evagination through a weak gut wall. Diverticuli of the descending colon and sigmoid (of unknown etiology) have an incidence of 5 per cent in adults. Inflammation or bleeding of the diverticuli may occur, and present common medical problems.[38,94] Diverticuli of the duodenum and jejunum also occur,[39] and are often asymptomatic.

### 2. Malrotation or nonrotation

These conditions arise either from incorrect movement of intestinal loops about the axis of the superior mesenteric artery, or abnormal replacement of the gut returning from the yolk stalk. Such forms of dysrotation are to be distinguished from situs inversus (a transposition of right for left).

### 3. Umbilical hernia

This may result from either a persistence of the intestine in the umbilical stalk, or a secondary herniation after withdrawal has taken place.

### 4. Imperforate anus

Failure of the cloacal membrane to rupture at the end of the eighth week of development, results in a body of tissue between the proctodeum and the future rectum. More recently, a theory of the failure of the rectal opening to migrate has been proposed.[89] After the newborn infant with an imperforate anus has had an opportunity to swallow air, an X-ray with the child held inverted may reveal the extent of the soft tissue imperforate mass between the skin and the gas filled bowel.[106]

### 5. Stenosis or atresia[99,109]

Most commonly occurring in the duodenum, these anomalies are due to a defect of the gut primordium, or follow from a retention of the usually temporary fetal occlusion, or intrauterine intussusception.[104] Of particular interest is the duodenal atresia occasionally found in cases of mongolism.[40]

Other congenital anomalies of the intestinal tract which are of importance either because of common occurrence or bizarre manifestations, include the following:

Partial or complete bands
Gastroenteric anomalies with absence of the spleen[41,42]
Dorsal intestinal fistulae[43]
Duplication of gut segments
Ectopic tissue (such as gastric or pancreatic tissue in Meckel's diverticulum[44] or colonic diverticuli)[80]
Polyps
Abnormalities of Kerckring's valves[105]
Interposition of large bowel between liver and diaphragm (Chilaiditi's syndrome)[45,95]
Duodenal obstruction due to annular pancreas[46]
Duodenal obstruction due to superior mesenteric artery[47]
Defects of intestinal lymphatics (in bowel villi, serosa and mesentery).[48,82,83]

## GENETICALLY DETERMINED ERRORS OF THE INTESTINAL TRACT

Information regarding the genetic control of intestinal structure and function is quite inadequate. Knowledge of gene-intestine interactions exists only in terms of inherited errors, and even these are few in number. It is possible that a majority of genetically determined disorders of the gut are incompatible with survival; careful examination of the intestinal tract of stillborn infants and those dying with congenital anomalies might be rewarding. We may proceed to examine data on a number of (apparently) genetically determined errors which involve the intestinal tract, keeping in mind the fact that few family pedigrees are available.

| DISEASE | COMMENT |
|---|---|
| *Celiac Disease* | The risk for siblings of affected patients is 13 per cent.[84] An unconfirmed report states that ethanolamine-phosphoric acid is involved in the defect.[49] This compound occurs in the small gut of animals,[50] and its presence in the urine can be detected by chromatography (which may have use as a screening test). |
| *Multiple polyposis of colon* | Majority of cases may be due to a dominant gene with reduced penetrance. About one-third of the cases are sporadic, and could represent new mutations. Rarely, polyps extend into the small intestine. There has emerged a concept of 'familial adenomatosis' with polyps of the colon as but one manifestation of a multi-faceted error.[51,52] |
| *Peutz-Jeghers Syndrome* | The syndrome consists of multiple intestinal polyps associated with pigmented spots on the oral mucosa, and often on lips and fingers. Inheritance is possibly by means of a dominant gene with reduced penetrance. A number of variants may be related, such as the syndrome described by Cronkhite and Canada.[53] Rarely are the polyps malignant.[100] |
| *Visceral situs inversus* | At autopsy, the incidence of this disorder is about 1 in 7,500. Siblings of affected individuals run a greater risk than the general population. There may be no associated symptoms, or they can appear late in life.[54] There is frequent association of situs inversus with bronchiectasis and sinusitis (which is termed Kartagener's syndrome). Multiple forms of the latter syndrome are known.[55] |
| *Hypertrophic pyloric stenosis* | Both genetic and postnatal factors have been discussed as etiologic factors.[85,86] Incidence is approximately 3 per 1000 births. Increased risk for siblings noted, and cited as being more frequent in the first born. |

| | |
|---|---|
| *Hemochromatosis* | An intestinal error permits excessive iron absorption; perhaps inherited via recessive genes (see chapter on Supra-absorption). |
| *Fibrocystic disease of the pancreas* | Occurs as frequently as 1 in 1000 births. Inheritance is perhaps recessive. Some cases present with meconium ileus, and the meconium contains large amounts of protein derived from serum albumin.[15] |
| *Wilson's disease (hepatolenticular degeneration)* | Most likely recessively inherited, this defect involves diminished ceruloplasmin synthesis (the blood enzyme which carries copper). One of the errors associated may be an excessive absorption of copper by the gut.[56] (See chapter on Supra-absorption.) |
| *Familial idiopathic hyperlipemia (Buerger-Gruetz disease)* | The syndrome is characterized by abdominal pain, perhaps from distention of intestinal lymphatics by elevated lipids, or from pancreatic involvement.[57] Inheritance is recessive.[58] |
| *Megacolon and Hirschsprung's disease* | About 1 birth in 25,000 is accompanied by megacolon, with an incidence as high as 20 per cent in male siblings. At least three entities (perhaps variants of the same basic process) are involved.<br>A. 'Classical' Hirschsprung's disease in which there is colonic achalasia accompanied by absence of ganglia in the myenteric plexus.[59,60]<br>B. A more severe form which affects the small gut as well as the large.[61,62]<br>C. An unusual type in which there occurs achalasia despite the presence of ganglia in the myenteric plexus.[63]<br>The development of the intrinsic visceral ganglia has been discussed.[64] |
| *Hereditary alactasia* | A recently described syndrome of failure to thrive, due to inability of intestine to hydrolyze lactose.[107] |
| *Hartnup disease* | Likely defective intestinal absorption of tryptophan. The nonabsorbed tryptophan is largely attacked by intestinal bacteria.[110] |

## BLOOD GROUPS AND GASTROINTESTINAL DISEASE

The first reliable study of the possible relationship of genes determining the ABO blood groups to gastrointestinal disorders was reported by Aird and coworkers.[65] These investigators showed a significant increase in the incidence of blood group A in cases of carcinoma of the stomach, when compared with a control series of patients from the same hospitals in Great Britain. The same association has been noted in cases from Denmark[66] and Switzerland. A follow up study by Aird[67] revealed a statistically significant difference between the blood group distribution in males and females with gastric carcinoma. The men had an excess of group A, whereas the women possessed a normal blood group distribution.

The distribution data has also been interpreted as indicating that persons with blood group O are 35 per cent more likely to develop peptic ulceration than individuals of other blood groups.[66,67,68] Additional studies reveal that blood group O is more common in duodenal ulcer patients[69] and those with nontropical sprue,[70] but not in cases of gastric ulceration. Woolf[71] discussed the technique of estimating the relationship between blood groups and disease, and his paper as well as that of McConnell[72] may be consulted for additional data. McConnell also related the incidence of gastrointestinal disease to a lack of the ability to secrete blood group antigens into body fluids (the secretory ability is inherited as a Mendelian dominant—see chapter on Production and Translocation of Gastrointestinal Enzymes).

## FUNCTIONAL ANATOMY

The accurate diagnosis of gastrointestinal disorders often depends upon an understanding of the geographic positioning of the regions. Gross anatomic locales are not entirely rigid, but knowledge of their approximate relations can be applied to an interpretation of signs and symptoms (see particularly Cope[73]). For instance, non-penetrating injuries to the abdomen should alert the diagnostician to intestinal damage, as the gut can be pressed against the vertebral column and crushed.[74]

Since the researches of Cannon on the use of x-ray contrast media for visualization of the gut, it has been recognized that the intestinal canal has a remarkable degree of mobility. Hence it can 'reef' up on itself, causing variations in intestinal length[75] and difficulties in exactly locating a bleeding site. Such points on gross anatomy have been well covered elsewhere. We are not amiss, however, in reviewing certain aspects of the microscopic anatomy of the gut. It is the individual cells (especially those lining the lumen) which produce enzymes, absorb nutrients, and are charged with the ultimate tasks of the intestine.

The alimentary canal possesses great metabolic activity and its components have a limited life span; it thus becomes understandable why intestinal cells are so readily affected by a lack of essential metabolites in the diet. The rate of renewal of gastrointestinal epithelium has been studied principally by two techniques: (1) Histologic localization of tagged $P^{32}$-deoxyribonucleic acid;[76] and (2) Use of colchicine to stop cell division at metaphase.[77] By the use of the latter technique, Stevens and Leblond reported that nearly 6 per cent of rat gastric surface epithelial cells entered mitosis in 4 hours. Hence 100 per cent of the cells would have 'turned over' in 68 hours. Mucous neck cells were renewed at a lesser rate, about 2.5 per cent in 4 hours. Stevens and Leblond concluded that a significant number of cells must be lost by extrusion or other means (a considerable return of exfoliated cells can be achieved by gastric and colonic lavage and this has been employed in the cytologic diagnosis of gastro-intestinal tumors). Changes in the gastrointestinal tract with age have been reviewed by Andrew and Andrew,[78] and cell population kinetics are known.[108]

The microscopic structure of the gut may be considered in terms of four tubes, one within the other (mucosa, submucosa, muscular coat, and serosa). Mucosal cells are supported by the fibrous lamina propria, and are thrown into a number of coarse, closely placed transverse folds (plicae circulares). Plicae are permanent anatomic structures which are composed of both mucosa and submucosa; their distribution is from the second part of the duodenum to the ileum.[105]

Due to multitudinous villi, the mucosa of the small intestine appears velvety to the naked eye. Villi are most numerous in the duodenum and jejunum where, sitting on the plicae, they project into the lumen. During distention of the gut, the villi tend to disappear. Aside from a transient appearance during the early fetal period, villi do not normally occur in the large gut. Between the villi can be located openings which indicate the intestinal glands (crypts of Lieberkuhn). These are anatomically simple tubular structures, but their physiologic role is not simple, since they perform multiple tasks. In the duodenum, particularly its upper portion, are located the larger glands of Brunner,[101] which occupy the submucosa.

Small lymph nodules are scattered over the mucous membrane of the small gut, and some of them ('solitary nodules') extend into the submucosa. Within the ileum, aggregations of lymph nodules occur; these are designated Peyer's patches (their major axis follows the long direction of the gut on a line generally most remote from the mesentery). The submucosa is composed of loose tissue which contains a vascular network and the submucous nerve plexus of Meissner. Between the mucosa and submucosa is located a thin muscularis mucosae (whose fibers may perhaps be arranged in outer longitudinal and inner circular bands). The muscularis proper, beyond the submucosa, has its fascicles distinctly arranged in outer longitudinal and inner circular fashion. Between the muscles are scattered divisions of the myenteric (Auerbach's) plexus. The outermost layer of the gut (serosa) has a mesothelial covering, except in the retroperitoneal areas of the duodenum.

The characteristic layers of the alimentary canal (mucosa, submucosa, muscularis, serosa) are also found in the large gut. However, the large bowel lacks the plicae, Peyer's patches, and villi of the small intestine. The inner muscular coat is arranged circularly, while the outer longitudinal fibers are gathered into three bands called the tenia coli. The appendix may contain fat cells in the submucosa as well as dense lymphatic aggregations, and its circular and longitudinal muscle bundles are generally close together. The rectum has its individual morphology with transverse folds (Houston), rectal columns, sinuses and valves, and transitional epithelium until the skin surface is reached. In the lower portions of the rectum, the serous coat is absent, and the circular muscle thickens to contribute to the internal sphincter.

Only a few cell types comprise the layer which lines the alimentary lumen, and they are charged with the tasks of absorption, secretion, and alteration of nutrients.

1. Epithelial cells. These vary from columnar to transitional in appearance. No secretory activity has been assigned to them, and they may be the units which perform absorption. Electron micro-

scope studies[102] reveal the luminal surface of epithelial cells to be thrown into many folds ('microvilli') which may aid in the absorptive process.

2. Goblet cells. These become more numerous as the large bowel is approached. They appear both on the surface and in the crypts of Lieberkuhn. Their function is probably mucous secretion (q.v.). The recent development of a fluorescence technique for demonstrating mucous in the gut[87] may enable the life cycle of these cells to be traced.

3. Paneth cells. Such units generally occur only at the base of the crypts. They are large, pink-staining or clear, and contain a fine reticulum in their cytoplasm. Paneth cells show cytochemical activity suggesting a secretory function.[81] By analogy to the secretory cells of other organs, they are perhaps responsible for producing enzymes used in digestion.

4. Argentaffin cells. These are also termed enterochromaffine cells. Their cytoplasmic granules show an affinity for silver (argent-) and chromium (chromo-) salts. The relationship of argentaffin cells to serotonin production is discussed in the chapter on Carcinoid Syndrome.

With this brief introduction to the morphology of the intestine, we may proceed to a discussion of the methods used in studying intestinal activity.

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## 2

# CLINICAL & LABORATORY TECHNIQUES

During life, the major portion of the intestine is inaccessible to direct observation. To overcome this hindrance, a variety of techniques, some of which are schematized in Figure 1, have been

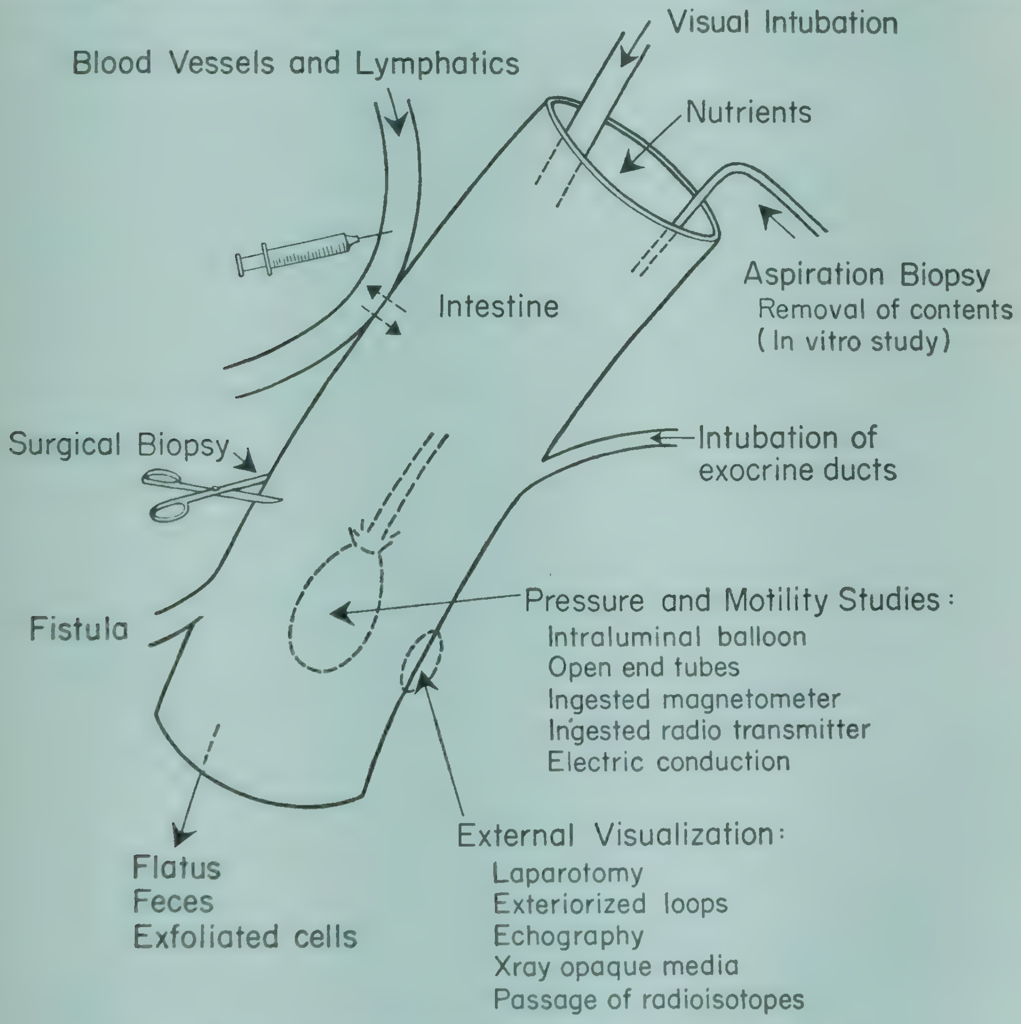
Visual Intubation
Blood Vessels and Lymphatics
Nutrients
Intestine
Aspiration Biopsy
Removal of contents
(In vitro study)
Intubation of exocrine ducts
Surgical Biopsy
Pressure and Motility Studies:
Intraluminal balloon
Open end tubes
Ingested magnetometer
Ingested radio transmitter
Electric conduction
Fistula
External Visualization:
Laparotomy
Exteriorized loops
Echography
Xray opaque media
Passage of radioisotopes
Flatus
Feces
Exfoliated cells

Figure 1. Methods of studying the intestine *in vivo*.

devised. We will consider each of these principal clinical and laboratory methods in turn.

### 1. Examination of feces

Study of the feces remains one of the simplest methods of assaying intestinal function. Ingestion of a dye or 'marker' and its recovery in the stool has been used to determine 'transit time.'[1] Analysis of feces for parasites and pathogenic bacteria, undigested food, intestinal and pancreatic enzymes, and bile constituents, can be performed with modest laboratory equipment. However, data as to what comes out in the stool must be interpreted in terms of the dietary intake; this has led to the balance study.

### 2. Balance Studies

The basis of these procedures is comparison of excreted materials with those ingested. An initial problem is to determine when all of the ingested nutrient, or its metabolites, has been passed per rectum. A simple, but occasionally unreliable, approach is to administer a 'marker' after the test meal; stools are then collected until all of the marker is passed. The number of markers which have been used is large: dyes (charcoal, carmine), x-ray opaque media, and radioisotopes. It is obvious that the marker should: not affect the gut, not be metabolized, freely admix with the stool. Use of a marker means that one is committed to save all stools until traces of the marker no longer appear. This can be a long and tedious task. Such a study also tells nothing of the exchange that is taking place across the intestinal wall. Thus cholesterol can be taken orally, and cholesterol recovered in the stool. There is no guarantee that an ingested molecule was one of those excreted; the use of radioisotopes to determine the extent of exchange is discussed in later chapters. A formulation of the basic equations for handling the problem of resecretion has been presented by Spencer.[2]

The use of inert indicators, as an approach to the problem of gastrointestinal exchange has recently been reported by a number of investigators.[3,4,50,51] The essential problem is as follows. It may require stool collection for a week to insure that all of an ingested substance has been excreted. If one specimen from the collection is lost, the entire procedure is invalidated. These difficulties of prolonged and exact saving of feces are obviated by the ingestion of the inert indicator such as $Cr_2O_3$. A representative stool sample is taken and analyzed for both the amount of indicator and the amount of substance under study. By extrapolation, it is then possible to arrive at the stool volume necessary to give full excretion of the indicator (and test substance). This is an application of the Fick principle, so successfully used in the study of blood flow, to the study of stool 'flow.' By use of a radioactive form of the material under study, it is possible to obtain some idea of the 'dilution' of the substance by resecretion, by measuring the specific activity in the stool, or the intestinal fluid.

Innumerable methods for gathering and preserving stool are on record. It is preferable, however, to analyze feces soon after passage, since nitrogen and sulfur may be lost on drying the specimen.[5] The longer stool specimens sit, the greater is the possibility that materials of interest will be lost because of bacterial action. Techniques are also available for collecting and analyzing flatus.[6]

In addition to the standard methods, 'segmental' balance studies have also been carried out. Long intestinal tubes, with multiple balloons, are capable of blocking off the lumen at a desired point;[14] thus, intestinal activity above or below a certain region (or between successive balloons) may be studied. The level at which the balloons reside is usually checked by x-ray. Not only can the long tubes be used to remove fluids from the gut, they may be employed in the 'opposite' manner and nutrients introduced into the intestine when oral feedings are not practical.[24]

### 3. Absorption Studies

Tests supposedly measuring absorption have enjoyed clinical popularity and often an uncritical acceptance. The principal objection to most of these procedures arises in determining precisely when absorption has taken place. If we define absorption as the passage of a substance from the lumen into mucosal cells, then we must follow the decline in intraluminal concentration to quantitate the process. This is admittedly difficult to do clinically. What is usually substituted is measurement of the blood level of the material at stated times after ingestion. The problems which may be encountered are enormous: (a) Bacteria may degrade the substance. (b) The test material may be taken into mucosal cells but not immediately released into the circulation (this is perhaps true of vitamin A[25]). (c) If absorbed and passed into the portal circuit, the material can be stored or otherwise altered by the liver before it reaches the general circulation (some experimental attempts have been made to bypass the liver [48]). (d) 'Recycling' of a number of absorbed substances across the mucosa is known to occur (cholesterol, water, bile salts, vitamin $B_{12}$).

In addition to these difficulties, if the test agent is not in simple chemical form, it may be altered by digestive enzymes and time will be consumed in the digestive process (hence starch tolerance and gelatin tolerance tests are poor indicators of absorption). It can be seen that there are few absolute criteria available for the quantitation of absorption. Most 'tests' of absorption are comparative; the subject is compared with a supposedly normal control.

Another approach to the study of absorption, which supplements blood sampling, is to collect the intestinal contents or feces to determine the amount not absorbed. Such a method is particularly useful when a radioactive nutrient is being employed, such as triolein-$I^{131}$ or oleic acid-$I^{131}$. This is, in effect, another way to perform a balance study.

### 4. Intubation

During gastroscopy an occasional glimpse may be obtained of the uppermost portion of the duodenum. Aside from this, and the portion of the large bowel visualized during sigmoidoscopy, intubation can not bring the intestine within visual range. Long flexible tubes have been passed into the intestine for other purposes, some of which are listed below.

Aspiration of contents and determination of osmotic pressure[39]
Suction biopsy of mucosa[7]
In situ pH recording[8]
Measurement of intraluminal pressure and motility[9,10]
Localization of bleeding area
Measurement of intestinal length[11]

Tubes and balloons, especially of the larger variety, often act as mechanical secretagogues or irritants; mobility and secretion of the gut may thus be altered by their presence. The fundamental fact that most substances within the lumen are foreign to the mucosa, and elicit a response, must not be overlooked. To overcome this objection, "indirect intubation" has recently been employed in studies of intraluminal pressures and intestinal mobility. In one procedure, a small magnet is swallowed and field variations resulting from the magnet's presence and motion are recorded by a sensitive detector.[12] A procedure with wider applicability depends upon recording signals emitted from a pressure-sensitive telemetering capsule which may be swallowed.[13] The latter method has shown jejunal pressures ranging to over 25 cm of water while intracecal pressures intermittently reached figures over twice as great.

The value of pressure-biopsy tubes, which may be used during life, is enormous. The devices of Shiner[7] and Crosby (Walter Reed Medical Center) permit samples to be obtained from various parts of the intestine. Hence diagnostic accuracy can be increased and the effect of medications followed serially.

### 5. X-ray Investigations

Roentgenography, employing radiopaque media or gases to make the intestinal patterns stand out, has been highly developed.[15] Mobility, the mucosal relief, and the rate of transit have been studied. Newer contrast agents of lower viscosity and greater water solubility[16] permit gastrointestinal investigations to be carried out, even if an obstruction is suspected, without great danger.

### 6. Fistulae

Experimental, standard surgical, and traumatic fistulae have contributed much to the study of the intestine. For instance, Grace *et al.*[17] extensively investigated the reactions of the colon by observing mucosa prolapsed from surgically produced fistulae.

The most widely employed experimental fistulae are those of Thiry and Mann-Bollman.

Thiry Fistula. A loop of small bowel is isolated from the remainder of the gut by two circular incisions, 15 to 25 cm apart. Continuity of the main channel is restored by an end to end anastomosis. The isolated segment has its nerve and blood supply left intact through the mesentery. One end can be closed by sutures and the other end brought out through the abdomen. Both ends may be sutured to the abdomen a short distance apart (Thiry-Vella fistula). There are many variants of these procedures depending upon the type of animal used.[18]

Mann-Bollmann Fistula. The distal end of an isolated ileal loop is anastomosed to another part of the small intestine (commonly the duodenum). The proximal end is sutured to the abdominal wall. Since peristaltic waves are usually incapable of passing in other than the proximal to distal direction, the experimental fistula prevents leakage from its external opening.

### 7. Blood and Lymph Analysis

Study of blood and lymph going to, and coming from, the gut, has contributed in increasing amounts to an understanding of intestinal activity. One of the simplest procedures, previously discussed, is to sample the blood stream following ingestion of a nutrient. In addition, the blood may be analyzed for gastrointestinal hormones, and for enzymes translocated from the digestive canal (see chapter on Production and Translocation of Gastrointestinal Enzymes).

Bollman and coworkers[19] made a significant contribution by developing techniques for the collection of intestinal lymph in experimental animals, thereby allowing fat absorption to be more closely followed.

Analysis of arterial blood entering the intestine, and venous blood leaving, can supply information not only as to absorption, but also concerning the utilization of substrates by the gut wall itself.[20] Thermocouples and other devices, employing the blood flow, have been used to measure heat production in the splanehnic area.[21] Additional aspects of these techniques are discussed in the chapter on Blood Supply and Gastrointestinal hemorrhage.

### 8. Morphologic Studies

In the past, sections of human intestine were obtainable only at operation or necropsy. Three developments have alleviated the paucity of material for microscopic study.

(1) The collection of cells exfoliated from the gut, and re-

covered by colonic lavage, has been developed into a reliable means of analyzing morphologic changes accompanying alimentary malignancies.[22] Biochemical studies on such cells would be a positive contribution.

(2) Lesions within reach of the sigmoidoscope may be biopsied and then subjected to various staining methods. This procedure has been of aid in the diagnosis of schistosomiasis,[26] amebic colitis and tumors. Obtaining tissue during life avoids the many artifacts which appear upon death of the host (especially if hemorrhage preceded death[29]). Following biopsy of the intestine, regrowth of the epithelium rapidly occurs and indications of the original site of the lesion may be lost. To circumvent this, Sauntry and Knudtson have described a technique for marking the gastrointestinal tract after biopsy.[38] The method consists of injecting an inert dye into the area; the size of the particles is large enough so that they stay in a limited region.

(3) As discussed, devices are now available for obtaining biopsies of the intestine during life by the application of suction through a flexible tube.[7] The ability to follow mucosal changes during the course of alimentary disorders should prove of value.

### 9. Other Clinical Procedures

Peritoneoscopy, the introduction of a tube through an incision in the abdominal wall, may occasionally provide information of value by permitting visualization of the intestinal tract during life.[52] Air introduced into the peritoneal cavity (pneumoperitoneum)[54] can be used for outlining the gut on roentgenograms, and peritoneal lavage is a recognized means of removing various metabolites from animals during uremia.[23] A number of other techniques for clinical evaluation of the gut are of as yet unproved value (such as intraluminal electrical conductance measurements,[27] pulsed ultrasound,[53] and intraluminal echography[28]). As an aid to clinical diagnosis of intestinal obstruction, a tape recorder for picking up peristaltic sounds has been introduced.[30]

### 10. Additional Laboratory Methods

Numerous investigators have freed sections of the gut from the main channel and then reestablished continuity with the segment 'reversed' (proximal and distal ends interchanged), in order to study the propagation of peristaltic waves. The gut has been variously denervated, purged, electrically stimulated, and subjected to such drastic procedures that the significance of the results obtained can not be judged in meaningful terms. A procedure due to Mann and Williamson,[31] involving diversion of the gastric contents to areas of the small intestine other than the duodenum, has been employed in studies of the genesis of peptic ulceration. Other rerouting techniques and intestinal 'short-circuits' have gained use during investigations of motility and absorption. The interesting technique of turning the ileum 'inside-out' has been used to absorb fluid formed in the peritoneal cavity.[32]

Evisceration, with or without removal of organs outside of the intestinal canal[40] has revealed a role played by the intestine in protein degredation. For example, the recovery of thyrotropin injected into rats was greater following evisceration-hepatectomy than in normal animals[41] (the kidneys have since been shown to also play a role in the process of thyrotropin removal [42]). Albumin labeled with $I^{131}$ increases in concentration in the small intestine of rats during the first sixty minutes following injection,[43] whereas in most other organs, the concentration falls after an initial peak. Anomalously, it has been reported that removal of the small and large intestine of the mouse accelerates catabolism of $I^{131}$-albumin.[44]

Experimental techniques for following absorption were implemented by Fisher and Parsons'[33] introduction of an in vitro apparatus. A dual circuit was maintained, separated by a section of intestine. The advantage of this procedure has been the ability to oxygenate the intestine and hence keep cells viable for long periods of time. Such a procedure and its modifications, allows experimental control not obtainable by in situ perfusion studies.[34] An important advance was made by Wilson and Wiseman,[35] who introduced the use of everted sacs of intestine. With the mucosa on the outside, the preparation could be oxygenated and kept viable while transport from mucosa to serosa was studied. A further refinement of this technique[45] has reduced the bulk of the preparation to test-tube size and provided a readily available means of following absorption in the laboratory. Experimentally, the passage of electrolytes across the gut has been studied by means of radioisotopes, and through the use of exchange resins which bind materials entering the lumen. The transport of ions has also been related to the development of electrical potentials by the intestine.[55] In addition, mechanical changes have been studied in the gut by means of strain gauges,[36] and peristalsis has been recorded from the isolated gut.[49,56]

The study of intestinal function on the cellular and subcellular levels has not been far advanced. Some of the difficulties encountered (bacterial contamination, rapid autolysis) are discussed in the chapter on Production and Translocation of Gastrointestinal Enzymes and Proteins. Methods for obtaining gastrointestinal mucosa relatively free of underlying tissue are discussed by Kroeger and Edwards,[46] and one technique is illustrated by Latner.[47]

From this resume of the techniques used in studying the intestine, we will turn to a discussion of intestinal function during life.

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# II

# INTESTINAL FUNCTION AND ITS MODIFIERS

# 3

# INNERVATION, PERISTALSIS AND DEFECATION

The alimentary tract is a long hollow tube surrounded by groups of smooth muscle (circular, longitudinal, and muscularis mucosae). Part of the explanation of gut motility, food propulsion, and defecation, is thus to be found in the properties of smooth muscles, their integration and control (see also sections on Embryology, and on the biochemistry of these muscles).

Intestinal smooth muscle differs from its striated (skeletal) counterpart in several respects, the most germaine of which are as follows.

1. The time elapsed between reception of an adequate stimulus and the resultant contraction is longer for smooth muscle than for striated muscle.
2. Sustained contraction (tonus) can be maintained when intestinal smooth muscle is isolated from the central nervous system.
3. The ability to undergo rhythmic contractions is also contained in the externally denervated smooth muscle preparation.
4. In vivo, muscles of the gut receive a dual autonomic innervation (sympathetic and parasympathetic).
5. Smooth muscle has a lower excitability and longer chronaxie than striated muscle, in spite of its ability to respond to thermal and chemical influences (in addition to stretching).

Evans[1] defined the tonus of smooth muscle as the resistance which its substance offered to extension. Thus, over a wide range, $T=F/L$, where tonus ($T$) is expressed in terms of the extending force ($F$) and the length of the fibers after extension ($L$). Within physiological limits, the force of contraction of intestinal smooth muscle is most likely dependent upon the initial length of the muscle fibers (better known as Starling's law, when applied to diastolic filling of the heart[2]). Hence, distention of the lumen by gas, by fluids called forth by saline cathartics, or by bulk, may initiate strong contractions. Tonus of intestinal smooth muscles is apparently maintained with only a slight energy expenditure, and is thus relatively free from fatigue. The persistence of tonus depends mainly on the intrinsic plexuses, although influenced by efferent nerves (as during vomiting [3]). Changes in tonus are also elicited by factors acting directly on the smooth muscles (particularly pH changes and anoxia). For example, both the tone and motility of the gut are diminished when the blood oxygen saturation falls below 12.8 ml/100 ml[4] (normal value=20).

Upon stretching the gut, the walls become accommodated to the new volume, and the tone of the encircling muscle differs but little from the initial value. This has been referred to as the 'postural tone' of the intestine. The increase in capacity may be due not only to a lengthening of the individual muscles, but perhaps also to a slipping of the fibers, one over another, so that the wall grows in surface but is reduced in thickness. Approximate values for the pressure-volume relationship within the human colon, which may be determined by the use of balloons or other manometers, are shown in Table I (adapted from Merritt et al[5]). Additional examples of colonic pressures and their interpretation in terms of mobilities are presented by Davidson and Bauer.[6]

TABLE 1

DATA INDICATING THE PRESSURE-VOLUME RELATIONSHIP WITHIN THE HUMAN COLON

| *Pressure in cm Water* | *Volume Accommodated by Colon (cm³)* | | |
|---|---|---|---|
| | *Normal* | *Hypertonic* | *Hypotonic* |
| 10 | 250 | 50 | 800 |
| 15 | 400 | 100 | 3,000 |
| 20 | 650 | 150 | > 3,000 |

Adapted, by permission, from Merritt, H. H.; Adams, R. D.; Solomon, H. C.: Neurosyphilis. New York, Oxford University Press, 1946. p. 267.

More recently, data has been obtained by the use of a small pressure-sensitive telemetering capsule which may be swallowed.[9]

## NERVES WITHIN THE INTESTINE

Situated between the circular and longitudinal muscle layers is the myenteric plexus (Auerbach). Another nerve plexus is to be found in the submucosa (Meissner), but ganglion cells are less frequent here than in Auerbach's plexus. Possible existence of other discrete nerve elements in the wall of the gut (particularly subserous) has been mentioned by several investigators. Whereas Meissner's plexus appears to be relatively free of involvement in disease states, the myenteric plexus is involved in a few pathologic conditions. As discussed in the section on Embryology, 'classical' Hirschsprung's disease is accompanied by a lack of ganglion cells in Auerbach's plexus and atonicity of a section of the bowel. Wyllie has summarized the clinical management of such cases.[7,8] A more severe form of Hirschsprung's disease involves the small intestine as well as the large. An interesting variant presents with achalasia despite the presence of ganglion cells in the myenteric plexus.[6] The myenteric plexus may perhaps also be involved during the course of pellagra (Winckel, W. E. F.: Docum. neerl. et indones. morbis trop. 3:100-104, 1951), and during Chagas' disease.[51]

Nerve fibers pass between the intestinal circular muscles to connect Auerbach's plexus and Meissner's plexus. Postganglionic fibers from the sympathetic system lead to the smooth muscles, glands, and blood vessels of the alimentary canal. The sympathetic innervation probably contributes to both the intramuscular and submucous plexuses. The situation is complicated by the fact that some of the nerve endings may be adrenergic and others cholinergic. Vagal fibers connect with Auerbach's plexus and likely augment movement. Ogilvie[10] has described colic of the large bowel following sympathetic denervation; the obvious inference is that the stimulating action of the parasympathetic system was unopposed after sympathectomy. However, vagotomy may result in spasm of the small intestine.[54] The problem of stimulatory and inhibitory innervation of the intestine is incompletely solved.

## INNERVATION OF THE SMALL INTESTINE

Fibers carrying impulses from the intestine to the central nervous system do not constitute true sensory pathways, since discrimination of the type of stimulus can not be made. The vagus (cranial nerve X) transmits afferent impulses from the upper small intestine (and in animals, 90 per cent of the fibers of the abdominal vagus are afferent[47]). A second afferent path takes fibers from the small and large intestine and reaches the spinal column via thoracic and lumbar nerves. The entry of these filaments into the central nervous system has been used as the basis for theories of referred pain. Splanchnicectomy, in man, is often followed by a diminution of abdominal pain, suggesting 'sensory' fibers in these nerves.[34] Lipkin and Sleisenger[24] can be consulted for data concerning the stimulus intensity and duration of visceral pain. In different species, variations on the main pattern of intestinal innervation may be present.[12]

Afferent nerves possibly form the inward bound arc of reflex paths affecting secretion and motility. Mediation of reflexes via the prevertebral ganglia has also been suggested.[11] Reflexes from one part of the intestine to adjoining areas play a role in both metabolic activities[32] and the propagation of peristalsis. Chemoreceptors are probably present in the gastrointestinal canal,[13,14] and the mesentery contains receptors sensitive to pressure.[15,16] In addition, intestinal vessels possess receptors which react to certain chemical stimuli.[17,48] Some evidence indicates that the discharge of intestinal afferent nerves is related to the state of digestion.[18] Intestinal afferent fibers have been noted to exert an effect on various somatic and visceral activities far removed from the gut,[28,29,30,47] and conversely, it is well recognized that a number of stimuli may reflexly alter intestinal activity.

Efferent innervation of the intestinal tract is from the autonomic nervous system. Proceeding mainly via the lesser splanchnic nerve, fibers of the sympathetic system pass to the celiac ganglion and thence to the superior mesenteric ganglion. These relay stations send post-ganglionic fibers to the gut, ending in contact with muscle fibers. The least splanchnic nerve contributes to the inferior mesenteric plexus (also see reference 52), which then sends filaments to the distal colon. Parasympathetic fibers to the small intestine are primarily from the vagus. The dual parasympathetic supply to the large bowel, from the vagus and the sacral nerve, is an unusual feature of this organ (the vagus innervates approximately the first half of the colon).

The colon may undergo pendulum-like movements as it shortens and elongates.[33] In animals, such movements of a 'segmenting' type can be shown to be myogenic. That is, they depend on the muscles and are not abolished by nerve poisons, such as cocaine and nicotine. It was demonstrated early in the century that contraction of the circular muscle fibers continued after they had been stripped from both the submucosa and the longitudinal muscles[19,20] (in other words, after they were isolated from most ganglion cells).

In contrast with the above segmenting movements (which may not be of importance in man), peristalsis appears to be dependent on intrinsic nerves. Peristaltic waves are initiated by local gut wall reflexes; this was first suggested by Bayliss and Starling[21] who found that sectioning the sympathetic nerves and the vagus did not abolish peristalsis, while cocaine to the bowel wall stopped such movements. More recent work has shown that the peristaltic reflex was abolished by asphyxiation or removal of the mucous membrane.[22] The reflex arc has been postulated to consist of two neurons: sensory fibers with processes leading to the mucous membrane and to motor neurons, and the motor neurons themselves, which innervate muscle coats. Additional aspects of peristalsis are discussed later.

The claim has been made that villi have a motion independent of the underlying anatomic structures, and that a hormone is involved in this activity (see discussion of Gastrointestinal Hormones); specific nervous control of the action of villi is unknown. The innervation of the muscularis mucosae may differ from the general pattern. King and Robinson[23] reported that the muscularis mucosae of both the large and small gut performed in similar fashion (although there are no villi in the large bowel), and were innervated by both adrenergic and cholinergic fibers.

An interesting but little understood entity is the abdominal manifestation of epilepsy.[26,27] During an attack of abdominal pain, the electroencephalogram shows an abnormal pattern. In some cases, the pain may be 'primary,' while in others, it is perhaps secondary to abnormal gastrointestinal motility. The nervous pathways involved are uncertain.

## INNERVATION OF THE LARGE INTESTINE

The three longitudinal bands of muscle which run the length of the large bowel (taenia coli) are shorter than the underlying colon. The gut is thus drawn into a series of sacculations, known as haustra. Between the haustra, the coat of circular muscle is said to be thickened. The makeup of intrinsic nerve plexuses is approximately the same in both the large and small intestine; they are less abundant however in the large gut.

Parasympathetic fibers to the large bowel originate from two distinct sources. 1. The vagus nerve. 2. Sacral segments II, III, IV, via the pelvic nerve (nervi erigentes, which has branches to the bladder detrusor and sphincter). Vagal fibers usually do not pass beyond the first half of the transverse colon; the parasympathetic supply to the remainder of the large bowel is drawn from the cholinergic pelvic nerve.

Sympathetic fibers to the proximal large bowel (cecum, ascending and transverse colon) arise from thoracic and lumbar segments of the spinal cord. The sympathetic nerves pass via the splanchnics and eventually relay through the celiac ganglion. Distal portions of the bowel (descending colon, sigmoid and rectum), are innervated by sympathetic nerves from lumbar segments I, II and III. The lumbar fibers pass primarily via filaments of the hypogastric (presacral) plexus, as well as the inferior mesenteric plexus (an offshoot of the celiac ganglion). These nerves are probably true adrenergic pathways. Stimulation of the inferior mesenteric nerves results in relaxation of the distal colon. Such fibers may exert a constant inhibitory effect on the colon, and increased activity has been reported following their sectioning. A somewhat similar effect can be elicited by spinal anesthesia, which paralyzes the lumbar outflow. Spinal anesthetics undoubtedly result in paralysis of both the lumbar and sacral fibers; in such a situation, the intrinsic plexuses assume control of gut movements.

If the parasympathetic pelvic nerve is severed, while the sympathetics are left intact, the wall of the distal colon relaxes and difficulty in emptying the bowel ensues. When division of the sympathetic nerves (which leaves the colon hypertonic) is followed by destruction of the pelvic nerve, still greater difficulty is experienced by experimental animals in emptying the colon. Tonic augmentation of the bowel probably also arises from spinal cord centers above the sacrum.

## SPHINCTER CONTROL AND DEFECATION

The large bowel may be considered to have three sphincters, (ileocolic, internal anal, external anal[53]), which effectively turn it into a closed segment. These sphincters have been spoken of as having "reciprocal innervation." That is, sympathetic fibers form the motor nerves to the valves, in addition to causing inhibition of the bowel wall (as they usually do). The sympathetic nerves thus have the dual responsibility of inhibiting motility and contracting the sphincter. Nervous control of the ileocecal valve is less certain, and the valve is not always competent.[50]

The internal anal sphincter also has sympathetic fibers which are stimulatory, while the pelvic nerve (parasympathetic) inhibits muscle contractions. Defecation may be viewed as a function of parasympathetic fibers which relax the sphincter and excite the bowel wall. The external anal sphincter differs from the other bowel sphincters in that it is composed of striated muscle. Although maintained in tonic contraction, the external anal sphincter is under voluntary control. Afferent impulses from the sphincters may aid in inhibiting movements of the colon.

The urge to defecate is usually correlated with rectal contractions. Since some contractions are not associated with an urge, a threshold for perception has been postulated (and this may rise with age [35]). Defecation is essentially a reflex which may be consciously aided or inhibited. A medullary center and a sacral augmentation center may be involved in the process. The medullary center is perhaps located in the vicinity of the respiratory and vomiting centers (it is recognized that dilatation of the anal sphincter stimulates respiration, and a stimulus to defecation may inhibit vomiting). The rectum, like the bladder, has a certain intrinsic mechanism and sectioning the sacral spinal cord is not necessarily followed by complete fecal incontinence.

A commonly quoted figure for the intraluminal pressure threshold to initiate defecation is 50 to 70 cm of water. The actual defecatory movement consists of a contraction of the colon accompanied by shortening of the taenia coli. The taenia are attached (via the rectal wall and perirectal fascia) to the rectococcygeus muscle which is anchored to the coccyx; hence with this point of leverage, they may exert considerable force. Such movements are coordinated with the action of voluntary muscles as the diaphragm descends, the glottis closes, and the abdominal muscles contract. The rectal intraluminal pressure at rest is about 25 to 50 cm of water. At the height of the straining movement, it may climb as high as 150 to 250 cm of water. The increased pressure creates a strain against the pelvic floor which is held in place by several muscles (levator ani, coccygeus, transverse perineal). The levator ani probably performs other functions such as drawing the anus upward over the fecal mass and aiding the rectal sphincter in resisting the evacuation reflex.

## MOVEMENTS OF THE ALIMENTARY CANAL

Movement of the alimentary tract is determined by the intrinsic mechanisms, the central nervous system, and chemical mediators. The hormone enterogastrone influences activity, and so does serotonin. Various foods have different effects on motility, perhaps due to their respective abilities to call forth hormones. Products of digestion and numerous medications also influence intestinal motion. Youmans has summarized data on the neurohumoral and nervous control of intestinal activity,[25] and Code presented a study of the motility of the intestinal canal of man based primarily on balloon studies.[31] Somewhat teleologically, intestinal movements have been divided into two types: those which mix the contents of the tract, and those which propel the contents forward.

Early in the century, Bayliss and Starling[21] formulated the "Law of the Intestine." This stated that stimulation of the intestine was accompanied by a contraction of the muscular coats above a food bolus and inhibition below the mass. In attempts to duplicate this work, many difficulties have been encountered. Henderson[36] was unable to demonstrate the relaxation phase in the guinea pig gut and a simple contraction ring was the only movement observed. Alvarez[37] also challenged the "Law of the Intestine" and pointed out that it had been formulated on the basis of work done by the drastically unphysiologic means of placing balloons in the denervated bowels of animals treated with castor oil. Alvarez found that electrical stimulation of the serosa of the rabbit's intestine was followed by contraction above and below the stimulated point, and concluded that the contraction wave extended caudally without preceding relaxation. Meschan and Quigley[38] recorded a small and inconstant decrease in intraluminal pressure preceding the contraction wave. They stated that the inhibition was real, but neither essential for, nor of importance to, propagation of the wave.

The natural stimulus to peristalsis is distention, and depends upon a reflex within the gut wall. Transmission of peristalsis is selective as far as direction is concerned. For the most part, the gut is incapable of transmitting waves in the oral direction (there do occur minor 'antiperistaltic' waves in the duodenum and the region of the ileocecal valve). Alvarez hypothesized that the direction of propagation of peristalsis was determined by a 'metabolic gradient' with the oral end of the digestive tract being most active metabolically.[39] It was reasoned that conditions which reversed the gradient allowed peristalsis to pass in the oral direction (the limitations of this concept are discussed in the chapter on Luminal Cell Metabolism). If an area of the gut is resected and then reanastomosed but reversed in direction, food will be pushed through by the healthy region above, if the reversed segment is small enough. After survival of the animal for a sufficient length of time, the reversed segment 'adapts' and propels the bolus through normally.

There is some evidence that materials in the gut are transported in a spiral fashion,[40] perhaps due to placement of the muscles; the rotation is in an anticlockwise direction. The distance carried by a peristaltic movement is variable. Some die out after traversing only a few centimeters, while at other times, an almost pathologic 'peristaltic rush' may sweep the length of the tract traveling at a speed of from 2 to 25 cm/sec or faster. A strong cathartic may initiate a rush that travels almost from cardia to anus. A rush in the small intestine may be followed by one in the colon. The gastrocolic reflex, whereby an infant defecates while eating, is well known. Slower peristaltic waves (1 to 2 cm/sec) may move large masses down the digestive canal and can originate at any point in the alimentary tract. In addition to tonus, peristalsis, spiral rotation, and possible movements of the muscularis mucosae, the previously mentioned segmentation[41] and pendular movements may perhaps be of importance.

## INTESTINAL TRACT AND VOMITING

Vomiting (emesis) is of two varieties. The 'regular' type occurs through the use of diaphragmatic muscles and can occur after gastrectomy. A 'regurgitant' type of vomiting occurs in infants and is perhaps due to sphincter laxness and slight pressure differences.

The physiology and pharmacology of vomiting have been well summarized by Borison and Wang,[42] and a clinical discussion is presented by Cummins.[49] The central emetic mechanism consists of two distinct areas: a chemoreceptive trigger zone and an actual vomiting center.[43,44] Vomiting may be initiated by stimuli outside of the gastrointestinal tract (as during motion sickness),[45] by stimuli from the gut,[44] and by agents which affect both the gut and the vomiting center (such as uremia). Chlorpromazine significantly delays the onset of vomiting in complete high intestinal obstruction, due to its effect on the chemoreceptor trigger zone of the brain,[46] and should be used with caution when intestinal obstruction is suspected.

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# 4

# COMPOSITION AND METABOLISM OF CELLS COMPRISING THE INTESTINE

THE TERM 'alimentary tract' is a catch-all which includes the mucosa, submucosa, muscle layers, and investing mesenchyme of a diversified tube. Further, the layers themselves are a composite of multiple cell types. Thus any report on the composition and metabolism of the intestinal canal must be interpreted as the net effect of many different cellular entities. Only in recent years has information accumulated as to the functioning of individual cells in the gut, and even this is still scanty. Hence, the following discussion will be in terms of the entire canal or major segments, rather than in terms of distinct cells. Perhaps this is justified since each cell exists only in the company of others, and the whole is integrated both spatially and temporally to carry out the functions of digestion, absorption and elimination.

## Electrolytes and Water

The approximate gross composition of various parts of an adult male body have been reported by Mitchell and coworkers;[1] Table

TABLE 2

CHEMICAL COMPOSISTION OF AN ADULT HUMAN MALE [IN PER CENT]
(SELECTED SECTIONS; BODY WEIGHT ABOUT 70 KG.) *

| *Part* | *Weight as a Per cent of Total Body* | *Water* | *Ether Soluble Solids* | *Crude Protein (Nx6.25)* | *Ash* | *Calcium* | *Phosphorus* |
|---|---|---|---|---|---|---|---|
| Alimentary Tract | 2.07 | 79.07 | 6.24 | 13.19 | 0.86 | 0.0125 | 0.115 |
| Liver | 3.41 | 71.46 | 10.35 | 16.19 | 0.88 | 0.0102 | 0.148 |
| Pancreas | 0.16 | 73.08 | 13.08 | 12.69 | 0.93 | 0.0143 | 0.155 |
| Adipose | 13.63 | 50.09 | 42.44 | 7.06 | 0.51 | 0.0116 | 0.048 |

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*Adapted, by permission, from Mitchell, H. H.; Hamilton, T. S.; Steggerda, F. R.; Bean, H. W.: The chemical composition of the adult human body and its bearing on the biochemistry of growth. J. Biol. Chem. 158:625-637, 1945.

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TABLE 3

Electrolyte Composition of Mammalian Alimentary Tract*

| | *Constituents Per Kilo Fresh Tissue* | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Cl (mEq)* | | *Na (mEq)* | *Water (gm)* | | *Blood (cc)* | | *Fat (gm)* | | *Intracellular Water (Gm per kilo cells)* | |
| | *Rat* | *Rabbit* | *Rabbit* | *Rat* | *Rabbit* | *Rat* | *Rabbit* | *Rat* | *Rabbit* | *Rat* | *Rabbit* |
| Stomach | 63.0 | 56.1 | 32.6 | 737 | 802 | 7.7 | 5.9 | 36.7 | 27.5 | 529 | 671 |
| Stomach Muscle | — | 47.4 | 32.9 | — | 800 | — | 8.9 | — | 12.2 | — | — |
| Small Intestine | 49.0 | 45.0 | 50.0 | 677 | 806 | 4.1 | 7.4 | 49.6 | 7.0 | 547 | 703 |
| Large Intestine | 38.6 | — | — | 772 | — | 4.8 | — | 43.0 | — | 718 | 665 |
| Cecum | — | 24.5 | 46.9 | — | 833 | — | 2.1 | — | 13.6 | — | — |
| Colon | — | 43.9 | 56.1 | — | 748 | — | — | — | 44.3 | — | — |

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*Adapted, by permission, from Manery, J. F.; Hastings, A. B.: The distribution of electrolytes in mammalian tissues. J. Biol. Chem. 127:657-676, 1939.

2 is adapted from their data. According to these figures, almost 80 per cent of the alimentary tract is composed of water and the ether-soluble ('lipid') fraction is small compared with other organs.

Manery and Hastings,[2] reporting on the distribution of electrolytes in mammalian tissues, noted variations in the anion and cation makeup between different sections of the alimentary canal (Table 3). The composition of rat intestine differed from that of the rabbit; because of the apparent species differences, it may not be possible to directly extrapolate these figures to man. Electrolytes within the lumen can rapidly interchange with their counterparts in mucosal cells and other regions of the body. Over a long interval, net transport takes place from the lumen to the internal environment; this dynamic aspect of intestinal electrolytes is discussed in the chapters on Absorption, and Potassium Metabolism. During periods of reduced intake, serious electrolyte disturbances may occur as the outward movement of anions and cations continues without adequate replacement.

## Oxidation and Glycolysis

The respiratory quotient of intestinal mucosa is less than one[12] (Table 4, derived from data of Hawk *et al.*[3] and White and co-

TABLE 4

APPROXIMATE VALUES FOR RESPIRATION AND GLYCOLYSIS OF SELECTED RAT TISSUES*

| *Tissue* | *R.Q.* | $Q_{O_2}$ | $Q^{O_2}_{G}$ | $Q^{N_2}_{G}$ |
|---|---|---|---|---|
| Intestinal mucosa | 0.85 | 12 | 2 | 4 |
| Mucosa of duodenum | | 9 | | |
| Mucosa of jejunum | | 15 | | |
| Mucosa of ileum | | 5 | | |
| Liver (adult) | 0.5-1.0 | 10 | 0.5 | 3 |
| Liver (fetal) | 1.00 | 7 | 0.5 | 8 |
| Brain | 1.00 | 13 | 2 | 19 |

R.Q. = respiratory quotient = volume $CO_2$ evolved/volume $O_2$ consumed.

$Q_{O_2}$ = microliters oxygen consumed per hour by 1 mg. dry weight of tissue.

$Q^{O_2}_{G}$ = production of acid aerobically, measured in terms of the decomposition of bicarbonate (see below).

$Q^{N_2}_{G}$ = measurement of anaerobic glycolysis, expressed in terms of microliters of $CO_2$ produced by the action of acid evolved on bicarbonate in the medium, per milligram dry tissue per hour.

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*Adapted, by permission from Hawk, P. B.; Oser, B. L.; Summerson, W. H.: Practical physiological chemistry. 12th ed. Philadelphia, Blakiston, 1949. p. 297; and from White, A.; Handler, J.; Smith, E. L.; Stetten, DeW., Jr.: Principles of biochemistry. New York, McGraw Hill, 1954, p. 384.

authors[4]). The multiple activities of alimentary cells involve expenditure of all food substances, rather than carbohydrates alone. Cells lining the intestinal lumen must provide energy both for their own existence and for the mechanisms of digestion, absorption and motility.

While the inner border of luminal cells are in contact with the body proper, the external border is subjected to variations in temperature, acidity and so on, due to fluctuation of materials within the lumen. The mechanisms which allow mucosal cells to cope with such alterations are unknown. Their ability to carry out glycolysis anaerobically may be particularly impressive,[12] but apparently oxygen is required for normal functioning. Certain tissues have a depressed oxygen consumption during exposure to high glucose concentrations,[6] but whether the gut reacts similarly in vivo during glucose absorption is unknown. Oxygen suppresses apparent glycolysis in most tissues (the so called Pasteur effect); the Pasteur effect can be demonstrated in the rat ileum according to one report,[5] but not in the jejunum.[12]

All of the enzymes functioning in the tricarboxylic acid cycle (the main source of energy production in most cells) are known to exist in intestinal muscles and probably in mucosal cells as well. Crandall, Lipscomb and Barker[7] analyzed blood inflow and outflow from organs drained by the portal vein, and concluded that acetone bodies and glucose were used as energy sources. Werk and associates[8] described the production of acetone bodies in the splanchnic area and discussed methods for the quantitation of these compounds.

## Carbohydrates

Carbohydrate-phosphate complexes exist in the gut wall and have been thought by some workers to represent phosphorylated forms of absorbed monosaccharides. Enzymes are present, in the mucosa, for metabolizing glucose; a glucose-6-phosphatase can also be found. The intestinal wall can convert glucose to pyruvic and lactic acids[13] (but in most species, the major portion of absorbed glucose is likely passed along to the body proper without degradation). The intestine is capable of converting fructose to glucose[88,89] and parenterally administered fructose is partly removed by the splanchnic circulation.[9] Abdominal pain may result from the rapid administration of fructose solutions by the parenteral route,[10] but the cause is uncertain.

## Lipids

The lipid content of the gut varies depending, among other factors, upon the state of nutrition. There are also species differences in the lipid content of the gut. For example, the phospholipid content of rat gut is higher[14] than that of the cat.[15] The total lipid content can vary while one or two specific fractions are unaffected;[14] hence a mere statement as to gross lipid content may mask important alterations of specific components. Cholesterol also exists in the gut wall, being found in both free and esterified forms (these pools likely play a role in cholesterol absorption [85]).

Lipids pass not only from the gut lumen to the mucosa, but also in the opposite direction (and hence may dilute the specific activity of radioactive lipids placed in the lumen). Fecal phospholipids[16] likely arise from the interaction of the gut wall and ingested materials. Fractionation of stool lipid into its components is mentioned in the chapter on Absorption.

The classical divisions of lipids (neutral fat, fatty acids, cholesterol and its esters, phospholipids) fail to indicate the variety of these materials found in the gut wall. For instance, following administration of $P^{32}$-orthophosphate to the rat, by means of paper chromatography, 15 labeled compounds could be demonstrated, many of them lipid-phosphorus combinations.[17] Hahn and Hevesy[18] estimated phospholipid turnover by means of $P^{32}$ and concluded that it was greatest in the gut and liver. Also using radiophosphorus, Fishler and co-workers[19] demonstrated production of phospholipids in the intestine.

Materials such as choline complexes, which may be of importance in fat transport from the lumen, have only been partially characterized. It is probable that the intestine absorbs lipid fragments and resynthesizes lipids which are then released into the body. Hydrolysis and synthesis of fats occur in luminal cells,[20] and ketone bodies may serve as lipid precursors. Undoubtedly some of the lipids in the intestinal lumen are derived from the blood stream, and a significant portion of fecal fat consists of translocated blood lipid.

### Nucleic Acids

Studies utilizing radiophosphorus have indicated that there is a rapid incorporation of phosphorus into nucleic acids of the intestine. Essentially, the same results have been obtained by several groups of workers, and the gut mucosa appears to be a major site of nucleic acid synthesis. By the use of such procedures, Hevesy[22] determined that 25 per cent of the deoxyribosenucleic acid (DNA) of intestinal mucosa underwent turnover in a 24 hour period.

Intestinal mucosal cells are in rapid metabolic activity. Because of this, they are readily damaged by nutritional deficiencies or agents which interfere with pathways of metabolism. X-irradiation, which damages the intestinal mucosa, has been shown to degrade nucleic acids within the cells.[23] The uptake of nucleic acid precursors (purines and pyrimidines) into the gut mucosa is also active.[24] Metabolic antagonists which interfere with this process (folic acid analogues perhaps act in such a fashion) cause biochemical and morphologic alterations in mucosal cells.[25] The intestine may also play a role in the degradation of uric acid, the end product of purine metabolism, likely by bacterial action.[81]

### Proteins

The ability to synthesize proteins has been estimated by following the incorporation of radioactive amino acids into various organs. Using this simple parameter of nitrogen anabolism, the gut has been shown to possess the greatest facility for protein construction of any body organ.[26,27,28]

If the diet is changed so that it is isocaloric with the former intake, but contains a higher proportion of protein, several days may be required to reach a new nitrogen equilibrium. During the interim, considerable nitrogen is retained. Laboratory animals, subjected to such experiments, show an increase in the weight of liver, kidneys and intestine. Following a decrease in protein consumption, a loss of protein occurs from these organs. In starvation, nitrogen loss may not parallel total weight loss from the intestine, as lipids are even more labile than protein stores. Factors influencing protein construction in the gut are not yet delineated, but local reflexes may affect the process.[29]

Protein moieties are in rapid interchange with amino acids and hence with additional smaller molecules. When formate-$C^{14}$ is injected into the mouse, jejunal tissue incorporates a larger quantity than any other organ; some of the radioactive label can be demonstrated in amino acids, proteins and lipids.[30] The relationship of intestinal proteins to ammonia is discussed in the chapter on the Enterohepatic Circulation. Intestinal proteins can bind iodine, and contribute very slightly to the level of circulating protein bound iodine.[31]

**Phosphorus**

The essentiality of phosphorylation for carbohydrate absorption has been much commented upon, but not definitely proved; whether related processes occur during the uptake of other nutrients is unknown. Adenosinetriphosphate and creatine phosphate are likely among the energy sources driving the gut. The close linkage of intestinal phosphate to the state of nutrition is shown by the diminution of acid soluble phosphate during fasting,[32] depletion of necessary metabolites, or anoxia.[33] The possible role of phosphoethanolamine in the gut is discussed in the chapter on Malabsorption. The gut is one of the few organs which contains a phosphatase capable of splitting glucose-6-phosphate to glucose and inorganic phosphate. The significance of this enzyme is unknown.

**Sulfur Compounds**

The intestine contains glutathione (a tripeptide of glutamic acid, glycine and the sulfur amino acid cysteine [80,90]). Further, protein-bound sulfhydryl groups can be histochemically demonstrated in the rat duodenum.[34] The columnar epithelium is moderate in these groups, while mucous of goblet cells and connective tissue of the lamina propria are histochemically negative for protein-SH. By means of radioactive $S^{35}$, incorporation of sulfur into the gut from both sodium sulfate and dl-methionine may be demonstrated.[35,36]

### Extracellular Materials

The extracellular composition of the intestinal tract has been little studied. Spencer reported that the binding of mucosal cells to one another was not adversely affected by reagents which split sulfhydryl groups, but was altered by proteolytic enzymes and hyaluronidase.[37] Orekhovitch and Shpikiter noted procollagens (collagen precursors) to be present in the stomach of man but not in the intestine;[38] whether this is related to absorptive capacities is unknown. 'Collagen diseases' of the intestine have been reported,[39] and are discussed in a separate section.

### Mucin

Tenacious, viscous materials secreted into the intestinal lumen have been variously termed mucous, mucus, and mucin. Florey has discussed the secretion of these substances and their role in inflammation.[40] Recent work has centered on the biosynthesis of the mucins.[79] Interest has focused on such compounds because of their possible role in protecting the stomach against peptic ulceration[41] (mucous preparations have been used therapeutically as antacids). Glass has discussed the classification of mucous-like substances from the stomach and their possible sites of origin.[42] The secretion of gastric mucous is an energy requiring process which is independent of hydrocholoric acid secretion;[43] its exact relationship to intrinsic factor secretion has not been clarified. There is a possibility that abnormal mucous exists, since in tropical sprue, alimentary secretions behave differently than those of normal persons.[44] The production of mucous may be in response to local irritants;[45] nervous stimuli have not clearly been shown to be effective in eliciting its release. Increased outpouring of mucous occurs in the condition known as mucous colitis. The disorder is characterized by abdominal pain, spastic constipation, and intermittent bouts of diarrhea marked by the passage of masses of mucous.[91] Additional data on mucous colitis is contained in the clinical literature. Numerous papers on gastrointestinal mucous have been contributed by the laboratories of Glass, Hollander, and Florey; these may be consulted for basic information on the subject.

Mucous gives positive aldehyde tests after oxidation with chromic acid (Bauer) or with periodate (McManus-Hotchkiss [46]). Since these compounds stain metachromatically with toluidine blue, they are thought to be sulfated polysaccharides (and thus chemically related to heparin). Certain techniques, other than those above, do not stain mucous from all sites, but distinguish 'types.' 'True mucin' stains with mucicarmine and mucihematein and is metachromatic with toluidine blue (two subtypes are further distinguished on the basis of whether or not the material stains with aldehyde fuchsin). A substance termed 'mucoid' does not stain with mucicarmine or mucihematein, is not metachromatic with toluidine blue, but does stain with Best's carmine. Based on this classification, mucous of the stomach and Brunner's glands is primarily 'mucoid,' while that of goblet cells of the small and large intestine is 'true mucin.' Hicks and Matthaei[47] have described a selective fluorescence stain for mucin; whether it is applicable to all mucins is unknown.

## METABOLISM OF INTESTINAL MUSCLE FIBERS

The anatomic plan of the gut includes the muscularis mucosae, inner circular muscle and outer longitudinal muscle. Specific information as to the function and dysfunction of these fibers is slight. Hypertrophic pyloric stenosis, with its possible hereditary origin, has been mentioned previously. Gastrointestinal symptoms may occur in progressive muscular disorders,[48,49] and genetically dystrophic mice pass stools which are larger than normal perhaps due to disorders of the musculature.[50] Systematic morphologic and chemical studies of the intestinal musculature would be a positive contribution and might help explain such unusual findings as the pigmentation of these muscles in certain cases of malabsorption.[51]

Intestinal muscles are functionally responsive to a number of chemical mediators (histamine, parasympathomimetic agents, serotonin, posterior pituitary extract) in vitro, and a combination of such agents may be working in vivo. It has been mentioned that smooth muscles maintain intestinal tonus with only a slight energy requirement, and hence the process is relatively free from fatigue. Major contractions of these muscle fibers, however, are associated with the expenditure of high-energy phosphate bonds.[52] Smooth muscle of the rabbit intestine has been shown to utilize materials of the tricarboxylic acid cycle (such as succinate, alpha-ketoglutarate, fumarate, L-malate, citrate) for contraction energy.[53] Mechanical behavior of the gut during contraction has received some attention,[66] but further study is needed to delineate the normal chain of events.

Although the circular and longitudinal muscle groups of the intestine appear morphologically similar, it is not correct to assume that they react in the same fashion to all stimuli, or that they are functionally the same from pylorus to rectum. As pointed out in the section on Embryology, the circular muscle differentiates before the longitudinal fibers, and initial contraction rings pass in either direction along the gut.[54] Preparations of fetal guinea pig intestine react varyingly to epinephrine and norepinephrine, depending upon which section has the medications applied.[55] It has also been reported that certain choline antimetabolites inhibit muscle from rabbit ileum, but act like choline on jejunal muscles.[56] The conclusion to be drawn from such data is that the entire smooth musculature of the gastrointestinal tract does not act as a unified whole to all medications. Further examples of this phenomenon are given below.

1. Agents capable of exciting contractions of intestinal circular and longitudinal muscle (acetylcholine, muscarine, eserine) were ineffective in causing the longitudinal group to contract if the muscle preparation was denied glucose.[57] Addition of glucose or pyruvic acid restored the normal reaction. Less effective were three other carbohydrates (fructose, galactose, mannose), while lactate was beneficial to circular muscle.

2. The pituitary oxytocic fraction has been stated to relax the circular muscle layer, while constricting the longitudinal muscle of isolated rabbit small intestine.[58]

3. There may be a different distribution of cholinesterase between circular and longitudinal muscles.[59,60] Circumferential stretching of the gut has been stated to cause more of an increase in

acetylcholine content than longitudinal stretching[61] (however, it is possible that acetylcholine is produced or inactived by cells in the gut other than the muscles [62]) .

4. The circular and longitudinal muscles show different sensitivities to low temperatures.[78]

Biochemical factors affecting the minute-to-minute workings of intestinal muscles are not well understood. Probably many of the responses are initiated by the nervous system, but the full story of why travel through the esophagus takes only a few seconds while that through the colon requires many hours, has yet to be told. The metabolism of intestinal muscles is sensitive to hormone action. For instance, epineprine stimulates lactic acid production[63] and the rate of oxygen consumption.[64] Insight as to the modes of action of other materials affecting these muscles is still scanty;[65] in some cases, the type of response depends upon the dosage level.[21] The sensitivity of intestinal muscles to potassium levels is discussed in the chapter on Potassium Metabolism.

## ASPECTS OF THE GRADIENT THEORY

In the section on Embryology, it was pointed out that the cephalic region of the evolving gut differentiated more rapidly than the caudal region. Even at this early stage, differences appeared between regions of the gut. The distinct responsiveness of various segments of the evolving digestive tract suggests that these differences are carried over to the adult. Many activities appear to be going on at a higher rate in the upper reaches of the gut than the lower areas, with a gradually changing gradient in between. Thus the oral end of the gut has a greater concentration of creatine phosphate than the aboral,[32] and cyanide inhibits respiration of the upper reaches of the gut more readily than the lower regions.[68]

Such observations of the change of a number of parameters from cephalic to caudal ends of the gut have been seized upon to 'explain' many of the workings of the intestine. Thus Bayliss and Starling originated the 'Law of the Intestine' in which food propulsion was explained by a ring of contraction above the bolus, and an area of relaxation beneath. As we have discussed, this is an attractive hypothesis, but most likely is not the entire story in vivo.

Trendelenburg put forth a theory of 'proximal hypertonicity' to account for normal peristalsis occurring only in an aboral direction,[67] and Alvarez has advanced a 'gradient theory.'[11]

The gradient theory states that the basic factor in determining the polarity of intestinal peristalsis is the gradual diminution of metabolic activity of the intestine (including the muscles) that occurs from the pyloric sphincter downwards. The declining rate of activity was referred to as the metabolic gradient, and was supposed to cover such factors as rate of response, time required to respond, $CO_2$ production and oxygen consumption. Conditions which reversed or altered these processes would change the gradient and cause peristalsis to cease or proceed in the 'wrong' direction.

The theory accords with many clinical and laboratory observations, but by no means all. Thus, whenever a process is found which is more active in lower regions of the gut than the higher (such as absorption of certain materials), it must be shrugged off as being unimportant as far as the metabolic gradient is concerned. The gradient theory thus can not account for all of the facts, or predict new ones (and after all these are the two tests of the use of a theory). Much more work is needed before a single theory can be viewed as having satisfactorily described the processes of peristalsis and food propulsion.

## SOME ENZYMES OF THE INTESTINAL MUCOSA

The principal enzymes of the gastrointestinal tract are described under several chapter headings.* In this section will be discussed a number of enzymes of the intestinal mucosa which are demonstrable by histochemical techniques [46] or by in vitro action.

*Alkaline phosphatase* is demonstrable in small intestinal villi, but may be absent from intestinal tumors. *Acid phosphatase* is present in almost all gastrointestinal cells.[46] A number of esterases are present in the gut wall, including *lipases* (which hydrolyze fats), and *esterases* (which split simple esters). The levels of these two types of enzymes do not parallel each other in all sections of the gut wall (for example, the colon is stated to possess an active esterase). Some lipase can be demonstrated in cells throughout the gut, and perhaps a physiologic role is assumed when the body is deprived of pancreatic lipase.

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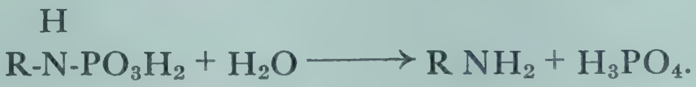
*See chapters on: Production and translocation of gastrointestinal enzymes, Enzymes and the Digestive process, Absorption, Malabsorption.

*Cholinesterase* has been discussed as being present in the intestinal wall. Gomori[46] states that *a phosphamidase* is present in gastrointestinal cells; *rennin* also has phosphamidase activity, splitting the bond between phosphate and an amide group:[82]



$$R\text{-}\overset{H}{N}\text{-}PO_3H_2 + H_2O \longrightarrow R\,NH_2 + H_3PO_4.$$

Histamine can be detected in the gut lumen following the parenteral administration of $C^{14}$-histidine.[69] Such a conversion can be affected by intestinal bacteria; whether the mucosa also possesses such an ability is uncertain. In the rat, only the lung and intestine are capable of degrading histamine.[70] Damage to the mucosa destroys the activity and histamine is absorbed;[71] this is rather conclusive that *histaminase* is present in mucosal cells. Such an enzyme may be of value in protecting the body against histamine produced in the lumen. Histamine is effective in contracting smooth muscles of the gut wall,[72] an action prevented by antihistamines. Interestingly, antihistamine agents may concentrate in the gut wall, and one of the pathways of their excretion is via the intestinal contents.[73]

Intestinal *xanthine oxidase* is an interesting enzyme as it contains molybdenum as an essential part of the molecule,[74] and in addition is dependent upon riboflavin for activity.[75,76] *Succinoxidase* of the rat intestine has been shown to be inhibited by fatty acids,[87] while lecithinase is activated by fatty acids.[92] The intestine may possess the ability to manufacture urea (a function usually ascribed solely to the liver) as both *arginase*[83,77] and *ornithine carbamyl transferase*[93] (components of the urea synthesizing pathway) have been demonstrated in the intestine. An enzyme, *hypertensinase,* capable of inactivating the pressor substance hypertensin, is also present in the intestine.[84] The possible relationship of *transaminases* to malabsorption is discussed in the chapter on Malabsorption.

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# 5

# SOME PHYSICAL FACTORS AND INTRALUMINAL GAS

THE LUMEN of the intestine is filled with varying quantities of food, digestive juices and gas. Pressure within the lumen is affected by contractions of the wall, and also by the pressure of gas in the surrounding atmosphere with which it is in more or less direct communication. The variation of gas volume within the lumen (assuming free communication with the atmosphere), is shown by the following figures:[1] 1.0 volume of gas at sea level occupies 4.0 volumes at 30,000 feet, and 7.6 volumes at 40,000 feet. The increased volume can cause discomfort and excess flatus. Approaching the problem from the opposite view point, it has been claimed that 30 mm Hg pressure exerted by rectal gas may produce distress.[2] A pressure of 210 mm Hg has been stated to result in rupture of the bowel in children (when produced by means of an enema),[23] while in the dog a cecal bursting pressure of 480 mm Hg has been noted.[24]

Gas escapes from the gut via either the upper or lower orifices. Rectal gas has been stated to bear little relationship to foods in the diet,[3] but certain individuals appear sensitive to specific items in their daily intake.[7] Flatus has been collected and analyzed as to both quantity and composition[4] (which varies widely). One estimate of the volume is 800 ml/day or 0.6 ml/min. The average composition of intraluminal gas (not necessarily identical with expelled gas) is given by Cantor[5] as: nitrogen 70-80 per cent, oxygen 1-10 per cent, carbon dioxide 4-18 per cent, hydrogen 1-6 per cent, and hydrogen sulfide 2-12 per cent. The diffusion of gases into balloons passed into the intestinal lumen depends on the nature of the gas and the material out of which the balloon is composed; hence samples collected this way may not be indicative of the true composition of gas within the lumen, unless equilibration is allowed to occur. Hydrogen sulfide, a normal constituent of alimentary gas, can damage intestinal balloons and lessen their effective life.[6]

The quantity of intraluminal gas is difficult to estimate in vivo, but attempts have been made,[7] since this information is valuable during measurements of body density. Three sources contribute to intraluminal gas.

1. There is passage of gas from the blood stream to the lumen.
2. Bacterial decomposition of food materials, particularly fermentation of carbohydrates, produces products such as $CO_2$, $CH_4$, $H_2$.
3. The greatest bulk of gas within the gut lumen comes from swallowed air. The estimate has been made that about three volumes of gas are swallowed for each volume of food.[8]

The turnover of intraluminal gases is probably many liters per day, as molecules enter and leave the blood stream. The proportion of gases in the mixture varies with their rates of diffusion across the gut wall.[9] Carbon dioxide diffuses most rapidly, and $H_2S$ also quickly moves across the gut membrane. The slowest rate is possessed by nitrogen, and hence its intraluminal concentration changes but little. Oxygen does not strictly follow the laws of diffusion, as it is bound to hemoglobin in the blood stream, which does not readily release oxygen for rediffusion into the gut. This is perhaps the point at which to stress that oxygen has two therapeutic actions during the treatment of intestinal obstruction. (1) Embarrassment of the blood supply to the gut results in hypoxia and deranged function; breathing an increased concentration of oxygen may halt this action and keep the gut viable until surgical intervention corrects the basic defect. (2) The principal gas in the gut lumen is nitrogen. By breathing a $CO_2$-$O_2$ mixture, nitrogen is slowly flushed from the body (including the alimentary lumen). Hence, distention decreases as carbon dioxide and oxygen, the only gases remaining in the lumen, can be readily absorbed.

Intraluminal gas has other clinical implications, such as in the meteorism and distress occurring immediately after eating. Swallowed air probably is the major cause, but such uncertain factors as 'food allergy' and 'abnormal peristalsis' have also been blamed. It is recognized that many individuals have a propensity for swallowing air, and then passing gas per rectum or per os. Gastric dilatation in such persons may be one of the sources of distress. Dilatation of the stomach can also occur following trauma to the abdomen, retroperitoneal hemorrhage, and other insults; the dilatation can be a serious clinical complication, but often responds to intubation. However, the 'routine' use of intubation, following surgery, to prevent dilatation, has been seriously questioned because of adverse effects.[10]

Intraluminal gas can be employed, during X-ray studies of the gut, to outline mucosal patterns. Air,[11] or preferably carbon dioxide,[12] is suitable for this purpose and is usually reabsorbed without difficulty; these gases are also employed following a barium enema to obtain 'double contrast' pictures of the bowel wall. Clinically, intraluminal gas can also be used in (a) estimating the thickness of tissue between an inperforate anus and the gut, (b) in diagnosing the level of intestinal obstruction. Two conditions confused with air within the bowel lumen are free air in the peritoneal cavity (pneumoperitoneum either induced therapeutically or occurring in disease states), and gas cysts in the intestinal wall (pneumatosis cystoides intestinalis [13]).

## OTHER PHYSICAL FACTORS

The gastrointestinal tract is in a relatively sheltered area of the body and is protected from thermal changes in the environment (but not from foods of varying temperatures); altered environmental temperatures may have some action on the intestinal absorption of carbohydrates.[22] During preoperatively induced hypothermia, the temperature of the gut is also lowered (which undoubtedly reduces activity; it is known for instance, that the nervous activity of the fish gut is sensitive to temperature [14]). Gut activity and response to medications depends not only on the temperature, but also on the speed with which the new temperature is attained.[15,16] At low temperatures, the response of the gut to medications differs from the response at 37° C. The role of the intestine in the maintenance of body temperature is uncertain.[17] A recent report has discussed the effects of hypothermia on intestinal secretion,[25] while rectal and esophageal temperatures have been recorded during induced hypothermia.[26]

## EFFECTS OF ELECTRICITY

Smooth muscles of the gut develop a potential of -40 to -80 millivolts.[18] The contraction of these muscles under an electric stimulus is a common observation. Following electrocution, it is claimed that there are no pathognomonic changes in the gastrointestinal tract, although direct intestinal lesions have been reported (Lagache, Gautier, Soulier: Lille Chir. 5:45-47, 1950). Metal-locating electric circuits have been employed for detection of metallic objects in the gut, and magnets can occasionally be used for their removal.[19]

## ANOXIA

In animals, occlusion of an artery and vein to the intestine, but with the nerve supply intact, is stated to induce a transient augmentation of breathing; on the basis of this, chemoreceptors have been postulated to occur in the gut.[20] Hind leg ischemia in animals, both lethal and sublethal, depresses gastric emptying,[21] and the shock accompanying the ischemia is worsened by feeding. Use of oxygen during intestinal obstruction, to reduce anoxia and permit the absorption of gases from the lumen, was mentioned above. The effects of anoxia on the composition and function of the gut are discussed in the chapter on Production and Translocation of Gastrointestinal Enzymes and Proteins.

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## 6

# PRODUCTION AND TRANSLOCATION OF GASTROINTESTINAL ENZYMES AND PROTEINS

## INTRODUCTION

IN MAN, during the course of a 65 year life span, if 2,500 calories were taken in daily, the gastrointestinal mucosa would absorb over 50,000,000 calories in the form of digested nutrients. The large majority of individuals perform digestion and absorption without difficulty, an accomplishment which reflects the adequate functioning of alimentary enzymes.

Certain disease states, however, are accompanied by errors of digestion or absorption, and focus our attention on the enzymes involved. Information concerning the production and translocation* of alimentary enzymes is far from complete, and a unified picture of the entire process can not be drawn. The present chapter will attempt to evaluate some of the data currently available.

## ENZYMES OF THE ALIMENTARY LUMEN

The origin of digestive enzymes[16,67,69,82] has been discussed in the section on Embryology. Developing enzyme systems are often sensitive to hormone action (for example desoxycorticosterone increases duodenal alkaline phosphatase in young rats and decreases the absorption of antibodies[179,67]) ; the enzymes may also respond to the presence of suitable substrates,[82] but integrated knowledge of the overall process is lacking.

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*By a translocated enzyme is meant one produced only in a distinct area of the body, and yet detectable in another region. The implication is enzyme transport from the first region to the second.

The alimentary canal and appendages, of a normal adult, secrete 8 liters of fluid into the digestive lumen daily.[54] This fluid is rich in protein; major contributions to the protein content are made by the pancreas[18] and small intestine. The average daily amount of protein entering the lumen of the digestive tract is about 35 grams (see chapter on Protein Loss).

This quantity represents half the daily protein intake, and may partially account for the negative nitrogen balance shown by individuals who have intestinal fluid removed during intervals of intubation. Such an observation has not received adequate clinical or biochemical study. The fact that protein loss in the stool is usually negligible suggests that the secreted proteins, or their digestive products, are reabsorbed.

Alimentary fluids can be sampled by intubation,[14] or drainage from experimental or accidental openings into the system.[12] Depending upon the level from which the fluid is withdrawn, and the number of cellular elements present, various enzymes implicated in the digestive process may be demonstrated (Table 5). Some of these enzymes, such as the phosphatases, may perhaps also function during absorption.

It would be most facile to assume that all the enzymes in a

TABLE 5
ENZYMES OF THE ALIMENTARY SECRETIONS OR MUCOSA
WHICH ARE INVOLVED IN THE DIGESTIVE PROCESS

| | |
|---|---|
| *Carbohydrases* | |
| $\alpha$-Amylase | Pancreas |
| $\alpha$-Amylase | Salivary glands |
| $\alpha$-Amylase | Small intestine |
| Maltase | Small intestine |
| Sucrase | |
| Lactase | |
| *Proteinases* | |
| Pepsin* | Stomach |
| Gastricsin | Stomach |
| Rennin | Stomach (animals) |
| Trypsin* | Pancreas |
| Chymotrypsin* | Pancreas |
| *Activator* | |
| Enterokinase | Duodenum |
| *Esterases* | |
| **Lipase | Stomach |
| Lipase | Pancreas |
| Lipase | Small & large intestine |
| Nucleases and Nucleotidases | Small intestine |
| Lechithinase | Small intestine |
| Acid phosphatase | Mucosal cells |
| Alkaline phosphatase | Mucosal cells, bile |
| *Peptidases* | |
| Carboxypeptidase | Pancreas |
| Aminopeptidases: | Small intestine |
| aminotripeptidase | |
| leucine aminopeptidase | |
| glycylglycine peptidase | |
| prolidase | |

*Secreted in the form of a proenzyme
**Probably has no physiologic activity

random sample of alimentary fluid represent active secretion by the gastrointestinal mucosa and its appendages. At least five sources for enzymes present in the digestive lumen can be visualized.

1. The diet contains numerous enzymes of both plant and animal origin. There has been little study of the action by, or on, these ferments during digestion in the intestine. The majority of reports in the literature have dealt with alimentary enzymes in fasting subjects, and hence, the dietary source of enzymes was excluded. There is a likelihood that 'foreign' enzymes would become inactivated upon reaching the acid environment of the stomach.

2. Intestinal bacteria produce multiple enzymes, which may be confused with ferments originating from the alimentary mucosa and its appendages. Thus, the small amount of a carbohydrate-splitting enzyme, reported in the juice of the large intestine, is probably of bacterial origin.[88] Urease, an enzyme demonstrated in the stomach of many species, has been shown to be due to bacterial contamination.[85]

Definitively stopping bacterial activity during studies of alimentary fluid or the mucosa is a difficult problem, since applied antibiotics inhibit some strains but not others;[51] there also remains the possibility that certain antibiotics may alter the production, activation, or functioning of gastrointestinal activities[174] and enzymes.[171] An interesting approach has been the study of digestive enzymes in germ-free animals,[89] but only smaller species have been so investigated. During actual life, however, digestion and absorption take place in the presence of bacterial enzymes. In the ruminants, enzymes from bacteria assume a major role in the degradation of ingested materials.[125]

3. The sloughing of injured and 'dead' cells from the mucosa carries enzymes into the alimentary lumen. Evaluation of the magnitude of this contribution is difficult,[162] as it requires information as to the regeneration rate of the mucosa and the degree of viability of enzymes within the sloughed cells.

Studies of the turnover rate of deoxyribonucleic acid,[72] of the incorporation of $P^{32}$ into the mucosa,[93] and of the inhibition of cellular division by colchicine,[144] have indicated a rapid rate of renewing cells lining the alimentary lumen (about 25 per cent per day). There is little evidence as to the functional activity of cells which leave the mucosa. Cells can be recovered from colonic washings;[52] these have been examined cytologically, but studies on their biochemistry have not been reported.

At one extreme of the ideas expressed, Florey and coworkers[47] were of the opinion that only enterokinase (a duodenal secretion that catalyzes the reaction: trypsinogen→trypsin) and perhaps amylase, were true secretions of the intestinal glands. Their argument continued that other enzymes in the lumen were due to sloughed cells, and that further digestion of absorbed disaccharides and peptides took place within the intestinal wall.[156] However, from carefully prepared intestinal fistulae, handled as little as possible, fluid can be obtained which contains multiple distinct enzymes. In vivo then, many enzymes are present in the lumen, and it may make little difference whether they are due to active secretion or to the sloughing of cells. As in many other 'either ... or' controversies, the fact of the matter may lie somewhere between the extremes. Histochemically, various enzymes (such as the phosphatases) are located at the luminal border of cells;[32,38,168] they may hence act at the surface,[128] being neither truly intracellular nor extracellular.

4. Blood and exudates entering the alimentary canal can contribute their enzymes to the luminal pool, but the fate of such ferments is unknown. This source of enzymes is negligible in healthy individuals, but during parasitic infestations[124] and disorders of the mucosa,[143] considerable quantities of blood and transudates can enter the lumen.

5. Finally, enzymes may be present in the digestive lumen because of active secretion by the mucosa or its appendages. Even when studying supposedly well characterized enzymes, difficulties may be encountered.

a. Species differences occur, and the enzyme composition of the alimentary contents from one animal may not resemble that of another species.[3]

b. The quantity of enzyme present is often a function of the diet. A prolonged high protein diet is followed by the outpouring of trypsin from the pancreas, whereas a diet rich in carbohydrate elicits a pancreatic juice with an elevated amylase content.[64] Each enzyme analysis must then be interpreted in terms of the particular stimulus employed, as well as the past dietary history.[164,165,166] Most enzymes of the alimentary canal are probably secreted at a basal level, with an increase in production or release following specific stimuli. Adaptive enzymes (whose production varies with the stimulus) apparently exist in the mucosa.[175,84,177] However, net digestive capabilities are limited by the specificity of enzymes which are present from birth or shortly thereafter.

c. The enzyme content of fluid taken from an alimentary fistula may change over a period of time. Enterokinase disappears from the intestinal fluid of dogs who have adapted to a fistula for about six months,[48] and from the fluid of horses after perhaps a longer period.[3]

## METHODS OF STUDYING PROTEINS AND ENZYMES OF THE MUCOSA

For the purpose of discussion we may, somewhat arbitrarily, subdivide the enzymes of the mucosa into two classifications: well recognized ferments which are common to most cells (such as enzymes of the tricarboxylic acid cycle), and enzymes which are involved in digestion and absorption (hence unique to this organ system).

Enzymes of the first type are often amenable to study by the use of manometric techniques,[150] but such methods have not been widely employed in analyzing the gastrointestinal mucosa. Three reasons are probably responsible for the lack of use of the conventional approach.

1. Microorganisms are ever present when alimentary tissue is investigated. Even though bacterial action may be considered to be minor during the short time interval of an experiment, appropriate controls should be run (such as the use of germ-free animals, or the prior administration of antibiotics to the animal). The danger in neglecting this control is well emphasized by the numerous reports of a gastric 'urease,' which is, in reality, due to bacterial action.[85]

2. As soon as the animal is killed, enzymes of the alimentary lumen (bacterial and those of the host itself), begin acting in con-

cert with intracellular cathepsins to digest the mucosa. Rapid preparation is thus essential, if meaningful results are to be obtained.

3. Aside from infrequently available sections obtained at operation, or from autopsies performed immediately after death, the intestinal mucosa of man was not available for study. The development, by Shiner,[139] of a long flexible tube for biopsying the intestine during life, will probably circumvent this difficulty.

Enzymes of the second type, those concerned with digestion and absorption, and hence unique to the gastrointestinal mucosa, or its appendages, have been investigated by a variety of methods. Enzyme systems are present for the synthesis of digestive ferments and also for the secretion of electrolytes into the lumen. This latter class of biologic catalysts has been almost entirely neglected; the only recent work is the demonstration that inhibitors of carbonic anhydrase diminish the outpouring of pancreatic bicarbonate[155] and gastric hydrochloric acid.[79]

Histochemical studies of the gastrointestinal mucosa have not been widely pursued. Gomori summarized some of the information on the enzyme makeup of the mucosa obtained by histochemistry,[58] and Barrnett[9] has demonstrated a wide distribution of protein-bound sulfhydryl groups in the intestine (interestingly, the application of sulfhydryl inhibitors to the gastric mucosa inhibits hydrochloric acid secretion, but not mucous production[77]). The possible study of cells exfoliated from the gut, by histochemical means, has been commented upon. There is certainly room for additional investigations of the alimentary canal by the techniques of histochemistry.

Incorporation of materials into the intestinal wall, in vivo, can be studied by analyzing arterial inflow and venous outflow. The method has yielded useful data as to the utilization of glucose and certain other metabolites by the alimentary canal,[29] but has yet to be extended to amino acids.

An interesting approach has been reported by Zavodskaya.[160] This investigator studied the uptake of methionine-$S^{35}$ by the gastric mucosa of guinea pigs. Entry of the labeled amino acid into the gastric mucosa was depressed following irritation of the duodenum, suggesting the transmittal of impulses from one area to the other.

However, explanted embryonic duodenum,[69] and homo-transplants of intestinal mucous membrane[71] both appear to function and produce their enzymes, indicating that they are essentially independent of direct nervous stimuli for maintenance.

Other workers have followed the incorporation of administered amino acids into the gastrointestinal tract,[15,49,117] but have not correlated this with enzyme production. At least part of the amino acid pool within mucosal cells 'exchanges' with other metabolites. Labeled formate-$C^{14}$ can be shown to transfer its radioactive tag to amino acids of the gut wall,[112] and $S^{35}$-sulfate becomes incorporated into the alimentary tract.[114]

Characterization of the proteins and enzymes of the gastrointestinal canal by antigenic studies has only been briefly mentioned in the literature. Schlamowitz prepared antibodies against dog intestinal phosphatase,[131,133] and noted that the enzyme was immunologically distinct from similar enzymes in the rabbit, rat and cow.[132] This investigator also reported the presence of intestinal alkaline phosphatase in the blood stream.[163] An interesting approach to the localization of gastrointestinal enzymes and their possible translocation, would be the preparation of specific fluorescent antibodies,[27,20] and the subsequent testing of the alimentary system, blood and other organs.

## ENZYME PRODUCTION BY THE GASTROINTESTINAL TRACT

Studies of protein synthesis have been hampered, because the end product is located intracellularly, rendering isolation and characterization difficult. Methods using disintegrated cells[53] give some insight as to the mechanism of production, but have not overcome the problem of contamination by other macromolecules. The technique of employing specific antibodies to the protein being investigated may offer an interesting means of circumventing this difficulty.[115] There are, however, proteins (or their precursors) which are liberated extracellularly after synthesis; among these are proteins of the blood stream and enzymes of the digestive tract. Such proteins may be isolated more readily than those which reside within cellular confines.

In order to follow the incorporation of amino acids into a protein, a digestive enzyme might well be chosen for study. Reasons for this choice are as follows.

1. The intestine takes up amino acids to a greater extent than most other tissues.[15,49,117] The incorporated amino acids (some of which must appear in enzymes), undergo a rapid turnover, and can be eluted from the preparation, creating a relative amino acid deficiency as desired.

2. Digestive enzymes elaborated by the bowel wall and its appendages are secreted into a hollow lumen, and hence can be easily collected relatively free of other proteins.

3. The system has a well defined protein (enzyme) as its end point. The quantity of enzyme present can be determined by its effect on the cleavage of a predetermined substrate.

These three points: rapid amino acid incorporation, collectability relatively free of other materials, and simple assay, suggest the potential usefulness of employing enzymes of the digestive tract in studies of protein synthesis.

After amino acid uptake, the next requirement for the production of an enzyme is probably the availability of an 'ordering' mechanism or 'template.' Such templates are usually genetically determined, but information concerning their function in the gastrointestinal tract is meager. Congenital errors of gastrointestinal enzyme production have been little discussed in the literature. It is possible that the paucity of material reflects the fact that these errors are not compatible with survival by the host. The logical source for cytological and histochemical studies of such errors, autopsies on stillborn infants and those with congenital anomalies, has not been pursued.

Fibrocystic disease of the pancreas, inherited as a simple recessive trait,[34] may be associated with defective intestinal mucous production in some cases. There is also recent evidence of altered mucous patterns in tropical sprue.[167] Aside from these reports, there is scant other evidence concerning the genetic control of gastrointestinal enzymes. Celiac disease, which involves probably more than one absorptive mechanism, has been stated to occur more frequently in siblings of affected individuals than in the population at large. In some cases of celiac disease, there has been reported an abnormal urinary excretion of phosphoethanolamine.[42] This material is a substrate for alkaline phosphatase, and a normal constituent of the small intestine of animals.[22] Additional reports extending or confirming this observation have not been noted.

Hemochromatosis, associated with excessive iron absorption from the intestine,[4] and Wilson's disease, in which excess copper absorption may occur,[161] have both been speculatively related to abnormal intestinal enzyme activity. Recent interest in the genetic relationship of the ABO blood groups to alimentary disorders[2] may shed light on the inheritance of the enzymatic processes involved. Other inherited disorders of alimentary function are known (such as the Peutz-Jeghers syndrome of intestinal polyposis and oral pigmentation), but their relationship to altered enzyme activity has still to be delineated.

In enzyme synthesis, the third factor that must be considered, in addition to amino acid uptake and the availability of a template, is a source of energy to carry out the reaction: amino acids→protein. Peptide bond formation requires the expenditure of energy, and the actual steps in the synthesis probably involve high energy phosphate bonds and phosphorylated intermediates.[50,75,142] Although we may infer that such compounds play a role in the intestine, only indirect evidence is available. Acid soluble phosphates of the gut diminish during fasting,[86] during the depletion of a necessary metabolite, and during anoxia.[87] In the course of the activities associated with absorption, the phosphorus content of the gut perhaps increases in certain fractions.[36]

## SOME LIMITING FACTORS IN ENZYME PRODUCTION

Rate limiting factors in the production of alimentary enzymes have not been clearly discussed in the literature. Anfinsen[6] has shown that in vitro the pancreas will incorporate $C^{14}O_2$ into its enzymes, and that crystalline radioactive ribonuclease can be isolated. An extension of this work to the intestine would be a valuable contribution, since it might allow the detailed study of rate limiting factors. Pancreatic slices can be shown to synthesize amylase under proper conditions,[158] but the net synthesis of intestinal enzymes by a preparation of gut mucosa has not been reported.

Enzyme synthesis is undoubtedly a complex phenomenon that depends upon the functioning of intact cells. In this respect then, it is unfair to separate cells from all normal stimuli and attempt to study the effect of one hormone at a time on enzyme manufacture. With this important limitation in mind, we can examine a number of reports in the literature.

In the discussion of the development of digestive enzymes, it was pointed out that adrenal steroids increase the intestinal concentration of alkaline phosphatase.[82] This enhancing action is also demonstrable in newborn animals[67] and possibly also in adults.[81] A number of papers have dealt with the effects of adrenalectomy on gastrointestinal enzymes. Tissieres[147] reported that adrenalectomy failed to produce alterations in the levels of intestinal acid or alkaline phosphatase. Adrenalectomy in the cat is accompanied by a decrease in the concentration of intestinal histaminase.[65] In rats, removal of the adrenal glands was associated with a decrease in intestinal adenosinetriphosphatase, while hexokinase was unaffected.[96] Such scattered observations can not yet be placed into a logical pattern. Castration of male rats has been reported not to affect the levels of acid or alkaline phosphatase in the intestinal mucosa.[148] An unconfirmed report has noted that parathyroidectomy in dogs is followed by an increased hydrochloric acid output by the stomach, as well as increased secretion of trypsin and chymotrypsin.[40]

Alterations of digestive enzymes are also discernible during increased or decreased thyroid activity. Hyperthyroidism in the dog is accompanied by an increased jejunal secretion of amylase, invertase, a peptidase, and a phosphatase.[41] Hypothyroidism can be accompanied by gastric mucosal atrophy,[57] alterations of the histologic appearance of the intestine,[10] and digestive disorders.

During dietary deficiencies, substances usually required in only minute amounts (minerals and vitamins), may become the limiting factors in gastrointestinal enzyme production. For example, rats fed a diet low in zinc show a number of abnormalities, including decreased activity of intestinal phosphatase.[123] This suggests that zinc forms part of the enzyme's prosthetic group or is essential for enzyme activation; other phosphatases have been shown to depend upon zinc for their activity.[149] Additional enzymes of the gastrointestinal tract which contain zinc are carbonic anhydrase and carboxypeptidase.[152] The trace element molybdenum may also be a rate limiting factor in enzyme production. Molybdate increases the intestinal concentration of xanthine oxidase when fed to weanling rats,[118] and the enzyme level falls during molybdenum deficiency.

There are at least two vitamin deficiency states which affect gastrointestinal enzymes. Pellagra, usually associated with a deficiency of other vitamins, in addition to niacin, is accompanied by a distortion of intestinal villi.[33] Diarrhea and impaired digestion may be principal clinical manifestations of pellagra. Thiamine deficient pigeons, with evidence of beriberi, have been reported to show increased levels of cholinesterase in the small intestine and decreased sensitivity to acetylcholine.[7]

Since enzymes are composed primarily of amino acids, diminished enzyme production should be expected during protein starvation. In the course of malnutrition, when weight loss has reached about seven per cent of the original weight, a transient depression of pancreatic enzymes occurs.[11] Minimal malnutrition may not seriously affect proteolytic activity,[97] but frank protein deficiency results in both anatomic[135] and functional changes. Within the past decade, the syndrome of kwashiorkor has been delineated and investigated. The condition consists of skin and hair changes, edema, alterations of the mucous membranes, hypoalbuminemia, and marasmus. The genesis of kwashiorkor is not entirely clear. It may represent the effects not only of protein deficiency, but also of an imbalance of amino acids, a diminished caloric intake, and vitamin lack. The duodenal concentrations of amylase, lipase, and trypsin are below normal in kwashiorkor.[146] Blood levels of a number of enzymes are also depressed, including alkaline phosphatase and cholinesterase.[136,31] Both of these enzymes occur in the intestinal tract; how accurately the depressed blood levels reflect alimentary activity is unknown. During the treatment of kwashiorkor by a high protein diet, the blood level of alkaline phosphatase first falls, and then climbs to a normal value[134] at the same time that full enzyme activity returns to the alimentary lumen.

## CONTROL AND RELEASE OF ENZYMES

The processes of digestion and absorption are exceedingly complex and it is to be expected that the control and release of alimentary enzymes would involve many factors. A few generalizations can be made.

1. Most digestive enzymes are secreted at a 'basal' level, and their outpouring into the lumen occurs in response to specific stimuli (such as nervous influences, hormonal factors,[63] or the presence of a substrate [64]). An increase in specific alimentary enzymes in response to administered substrates has been well documented for a number of species.[84]

2. Certain enzymes, particularly the ones involved in protein hydrolysis, are secreted in the form of an inactive 'proferment' or 'zymogen.' Upon activation, the zymogen becomes the functional enzyme. The activation process may depend upon other enzymes, or even on molecules of the active form of the enzyme ('autoactivation' [111]). The process of activation can involve the splitting off of a peptide fragment from the proenzyme; potentially at least, the fragment can recombine with the activated enzyme.[66]

Babkin[8] and Grossman[63] have summarized much of the literature on factors influencing the release of digestive enzymes (see chapter on Gastrointestinal Enzymes).

## FATE OF SECRETED ENZYMES

There is evidence that various enzymes have different effects on the intestine,[95] and hence a study of their fate within the lumen would be a positive contribution. At least three paths are open to enzymes entering the lumen, although the relative importance of each has not been quantitated.

1. A portion of the digestive enzymes are passed in the stool. This has proven of use in the diagnosis of pancreatic insufficiency.[140] Exocrine function of the organ is assumed to be normal if a certain titer of pancreatic enzymes appears in the feces. As usually run, the test consists of observing the liquification of gelatin (presumably by trypsin); bacterial enzymes often cause spurious positive results in adults.

2. Digestive enzymes in the alimentary lumen may be bound

to specific inhibitors and thus rendered ineffective. The best known example is a trypsin inhibitor found in soybeans and other foods.[91] The ultimate fate of the inhibitor-enzyme is uncertain. It could potentially be digested by other enzymes, passed in the stool, or possibly be activated once again.

3. Changes in pH can inactivate digestive enzymes. The prime example is the ineffectiveness of pepsin in the alkaline milieu of the duodenum. As the pH changes, potent enzymes are rendered ineffective and subject to the digestive action of other ferments;[176] thus, under alkaline conditions, trypsin digests pepsin. To what extent enzymatic digestion of other enzymes occurs within the gut has still to be determined.

## ALTERED ALIMENTARY ENZYME PATTERNS

Alterations in the pattern of mucosal or secreted enzymes due to congenital errors and ineffective diet have been mentioned. Variations can also occur in a variety of pathologic states (neoplasms, reaction to injury, effects of irradiation, anoxia) .

### Neoplasms

Of the multiple enzymes occurring in the alimentary mucosa and its secretions, relatively few have been studied during the process of tumor development, or in fully evolved tumors.

Pepsin and rennin are produced by the intact gastric mucosa, but vanish when the tissue becomes neoplastic.[62] In contrast, an enzyme depolymerizing a thymonucleic acid, as measured by viscometry, was practically unchanged in adenocarcinoma of the stomach and intestine as compared with normal tissue. Acid phosphatase can be found in cells of gastric carcinoma in animals, and the enzyme may be excreted into the gastric juice.[58] Greenstein states that the concentration of intracellular acid phosphatase in gastric carcinoma of rats is reduced to about one-half the normal value.[61] Alkaline phosphatase activity in the small intestine is greater than that of other body tissues.[61] The enzyme nearly disappears in gastric and intestinal carcinomas in rats.

One of the few enzymes studied in human gastrointestinal tumors has been $\beta$-glucuronidase. Fishman and Bigelow reported the concentration of the ferment to be increased in 37 out of 44 un-selected malignancies of the alimentary tract.[43] The tissue content of β-glucuronidase had no correlation with the histologic appearance of malignancy. The relation of the enzyme concentration within the tumor with the blood level was not reported. More recently, Wattenberg[178] has discussed changes in five enzymes in human intestinal carcinoma.

The use of enzymatic studies to determine the degree of malignancy of alimentary tumors has not been developed. Gomori[58] reported that a phosphamidase was present in large amounts in malignant epithelial tumors, and that all polyps of the colon gave a positive result (perhaps indicating their potential malignant character). Cells sloughed, or removed by biopsy, from tumors of the cervix have been studied by means of fluorescence techniques to determine the ratio of deoxyribonucleic acid (DNA) to ribonucleic acid (RNA);[151] this procedure has yet to be extended to cells of the gastrointestinal tract.

## Reaction to Injury

Injury to the alimentary tract can be accompanied by the 'leakage' of fluid from the vascular tree and extracellular spaces into the gut lumen. In addition, there may be changes in the functioning of luminal cells. At least two instances have been documented.

1. Irritation of the mucosa is accompanied by the outpouring of mucous.[159] This action is independent, in the case of the stomach, of acid secretion. Iodoacetimide and N-ethyl-maleimide (sulfhydryl inhibitors), inhibit acid release but permit mucous liberation when applied topically to the stomach.[77]

2. Lysozyme, an enzyme which lyses several strains of the saprophytic bacteria Micrococcus lysodeikticus, can be demonstrated throughout the large intestine and its secretions. Meyer and coworkers[102] discovered that the 24 hour fecal content of lysozyme was elevated in ulcerative colitis. The titer of the enzyme may exceed the normal range by a factor of 100; the analysis may be carried out on either wet or dried stool.[94]

The extra secretion of the enzyme appears to come from the bowel wall. The meaning of elevated fecal lysozyme is not clear. Lysozyme damages the colonic mucous membrane only under artificial conditions.[154] When given orally or injected directly into the superior mesenteric or celiac arteries, lysozyme produces no alterations in the colon.[99] This suggests that an elevated lysozyme concentration is not the cause of ulcerative colitis, but is rather the result of mucosal injury by the disease. This is supported by the fact that experimental wounds may have a high lysozyme content. Further, lysozyme does not liquify colonic mucous, and no substrate for the enzyme has been demonstrated in the normal or diseased colon. Lysozyme titers have been used to follow the course of ulcerative colitis,[80] and the stool concentration of the enzyme falls after steroid therapy is instituted. Estimation of lysozyme in the feces remains one of the few objective tests of colonic secretory activity.

### Effects of Irradiation

The rapidly metabolizing gastrointestinal mucosa is sensitive to ionizing radiations, and in this respect resembles gonadal tissue and certain elements of the hematopoietic system. The course of fatal whole body irradiation may be dominated by alimentary symptomatology (anorexia, nausea and vomiting, diarrhea, and bloody stools [78]).

Conrad and his group at the Naval Medical Research Institute summarized the literature dealing with gastrointestinal reactions to irradiation,[25] and have studied the treatment of this disorder.[26] Apparently the absorptive capacity of the alimentary mucosa is normally so great, that small doses of irradiation have little effect.[107] Specific studies on enzyme reactions are meager. Direct irradiation of the stomach (occasionally employed to reduce acid secretion) is accompanied by diminished uropepsin secretion.[120] X-irradiation of the intestine has been reported to result in a fall in pseudocholinesterase within 48 hours, while true cholinesterase was unaffected[17] (see chapter on Irradiation).

### Anoxia

The literature up to 1941, dealing with studies on the effects of anoxia on the gastrointestinal tract in vivo and in vitro, has been summarized by VanLiere.[153]

The anoxic gut reveals abnormalities of composition and function, suggesting that multiple enzyme systems are affected. Carbohydrate is not absorbed from the anoxic rat gut,[157] and the intracellular phosphate concentration falls.[87,28] In addition, both the tone and mobility of the gut are diminished when the blood oxygen saturation falls below 12.8 ml/100 ml[145] (normal value=20).

Specific enzyme studies on the anoxic intestine have often been inadequate and uncontrolled. There has been apparent failure to recognize that during anoxia or vessel thrombosis, damage occurs not only to the organ being investigated, but also to the formed blood elements and vessels themselves. For example, if a study were being made of intestinal phosphatases during anoxia, account would have to be taken of such enzymes in the anoxic region from erythrocytes,[1] leukocytes,[92] platelets,[105] and perhaps from the vessel walls.

Since Polowe's first report,[116] a number of cases of small bowel infarction in man have been reported in which the serum amylase concentration was observed to be elevated. An amylase is produced in the intestine, but until localizing studies (by means of specific inhibitors or enzyme antibodies) are available, it will not be possible to state whether this translocated enzyme arose from the intestine or from the pancreas.

Experimental small bowel infarction in dogs is accompanied by an increase in the serum glutamic-oxaloacetic transaminase concentration.[129] Vitally needed accompanying studies on the change in the concentration of the enzyme within the gut wall have not been reported. The effect of an anoxic episode on further functioning of intestinal enzymes is uncertain; a number of patients who recovered from small bowel anoxia (due to an embolus or thrombus) have developed malabsorption syndromes.[137,138]

## SECRETION OF OTHER PROTEINS

Fluid of the gastrointestinal lumen contains, in addition to digestive enzymes, a number of other macromolecular constituents. These components are normally present, and are presumed to be secreted by the mucosa. Among the macromolecules are:[119,104]

Intrinsic factor
Mucous
Blood group antigens (perhaps protein bound)
Antibodies

Intrinsic factor is produced in the stomach and perhaps also in the upper duodenum in certain species.[70] The mechanism of intrinsic factor release is unknown, and the amount produced probably varies among individuals.[39] Whether intrinsic factor recrosses the alimentary mucosa in combination with vitamin $B_{12}$ is uncertain.

'Mucous' is a term used to designate several diverse biochemical constituents, which can be differentiated by staining reactions and electrophoretic mobility.[58,55] The release of certain mucous fractions is probably dependent upon local factors.[46] Of interest is the condition known as 'mucous colitis,'[83] characterized by functional bowel complaints and increased mucous in the feces; this has suggested to clinicians that the release of at least one type of mucous may be under nervous control. The production of mucous has been studied by means of radioisotopes;[114] once synthesized, the possible fates open to these compounds are unknown. There is evidence that one type of mucous may recross the mucosa accompanied by absorbed proteins in young animals;[73] what contribution this makes to circulating mucoproteins, if any, has still to be determined.

The secretion of ABO blood group antigens into body fluids has been shown to be genetically determined, with the 'secreting' gene being dominant over its 'nonsecreting' allele.[130] Gastric secretion of the blood group antigens is a distinct event, separate from acid secretion[113] (just as mucous release is also independent of acid secretion [77]). Antigens of the ABO blood group can be demonstrated in the gastric mucosa (by their chemical separation [13]). Fluorescent antibody studies show the presence of blood factors $Le^a$ and H in both the gastric and duodenal mucosa.[56] Blood group specific antigens can also be recovered from meconium[141] but their significance is uncertain.

## 'NON-SECRETED' PROTEINS IN ALIMENTARY LUMEN

In addition to the previously discussed enzymes and proteins which are 'secreted,' the passage of various blood proteins into the alimentary lumen can be demonstrated or inferred in certain pathologic states (see chapter on Protein Loss).

A. The thick, ropy intestinal material found in meconium ileus, which accompanies cystic fibrosis of the pancreas, is principally composed of serum albumin.[60] The mechanism whereby this blood protein enters the lumen has still to be determined.

B. Individuals with giant hypertrophy of the gastric mucosa, in whom hypoproteinemia is a common finding, have been noted to 'leak' serum albumin into the gastric lumen.[21] Using $I^{131}$-albumin as a tracer, such individuals have been demonstrated to have a small body albumin pool turning over at an accelerated rate. In one case, the total exchangeable albumin pool was 122 grams, one third of normal, while the turnover was 17.6 grams per day (a value that is normal for an adult with an undepleted albumin pool).

Interestingly, in such cases of $I^{131}$-albumin 'leak,' there is no loss of the radioactive tag in the feces. It is probable that albumin within the lumen is digested and reabsorbed, but that production of 'new' albumin does not keep pace with the amount being degraded (which may exceed 8 grams per day [21]).

C. Certain patients with regional enteritis and ulcerative colitis also have small total exchangeable albumin pools. However, in contradistinction to the albumin leak in gastric mucosal hypertrophy, there is a 10 to 50 per cent fecal loss of administered radioactive albumin.[143] The small albumin pool may thus reflect prolonged fecal loss and inadequate replacement.

## PASSAGE OF AMINO ACIDS INTO ALIMENTARY LUMEN

Not only proteins, but also amino acids can be demonstrated in the alimentary fluid of fasting subjects. The presence of amino acids in the lumen may be due to either transport across the mucosa, or secretion into the juices of the accessory organs (liver, pancreas); the importance of these two sites in amino acid transfer has not been determined. Following administration of tyrosine-$C^{14}$, radioactivity appears in the intestinal lumen of mice,[117] but the nature of the material is unknown.

Studies performed in Germany on normal individuals[121] and subjects with a variety of disease states,[122] have shown that the duodenal juice possesses an individual distinctive composition with regard to the content of amino acids. This may represent inherited differences, since in mice, the excretory pattern of amino acids from the liver and kidney can be shown to be genetically controlled.[59] Adequate studies of this phenomenon would involve analysis of duodenal fluid amino acids in a number of families, to trace out any common patterns. The gastric juice also contains amino acids, and minimal qualitative changes have been detected in disease states.[100] Salivary fluid is another source of amino acids,[59] and adequate account would have to be taken of saliva that is swallowed, when analyzing fluids lower in the alimentary tract.

## ABSORPTION OF INTACT PROTEINS

Multiple enzymes and proteins pass from the blood stream and alimentary mucosa into the lumen. For completeness, mention is made that the reverse process (absorption of an intact protein from the lumen) can be shown to occur in special circumstances. Although such a process would allow the passage of proteins from the mucosa to the blood stream, it can not be considered true translocation since the proteins were not manufactured by the mucosa. There are two related aspects of the absorption of intact proteins.

1. Claims have been put forth that the uptake of unhydrolyzed proteins is related to infant allergy.

2. The absorption of globulins from colostrum may result in the young animal acquiring passive immunity to a number of infectious agents.[172] In the calf,[23] labeled globulins from colostrum pass from the intestinal lumen into lymphatics of the gut wall. During the absorptive process, particles with staining properties similar to colostral globulins can be demonstrated in the intestinal epithelium and adjacent lymphatics.[73,24] The route of absorption would seem to be from the lumen to epithelial cells, and thence to the lymphatics.[172] Colostral proteins absorbed into the mucosa are conjugated with a carbohydrate material (demonstrated histochemically[73]). The suggestion has been made that this material is one type of mucous.

The ability of young animals to absorb intact proteins diminishes with age. The suckling rat can absorb antibodies only during the first 18 days of life. Administration of desoxycorticosterone acetate to newborn rats diminishes their ability to absorb antibodies and increases the intestinal content of alkaline phosphatase.[67]

## TRANSLOCATION OF ALIMENTARY ENZYMES AND PROTEINS

First evidence for the translocation of protein materials from the alimentary canal was the demonstration of the hormones secretin and gastrin.[63] Indeed, the word hormone was coined to describe these products of the gastrointestinal canal, which were produced at one site and then transported by the blood stream.

Additional information concerning proteins translocated from the alimentary canal was provided by Morton and coworkers in 1943.[106] These investigators demonstrated that the completely thyroidectomized animal had a small, but persistent level of circulating protein bound iodine (PBI). The production of part of this protein was traced to the intestine. Thus, in two cases, the production and liberation of hormones and of PBI, the alimentary canal was shown to discharge protein products into the blood stream.

Evidence has also accumulated that the pancreatic enzymes, amylase, lipase and trypsin enter the blood stream, as well as pepsinogen from the stomach and enzymes from the intestine (alkaline phosphatase, transaminase, and perhaps acid phosphatase).

### Amylase

In 1929, Elman and associates reported that blood amylase was elevated during pancreatic disease.[37] This elevation was initially considered specific for pancreatic dysfunction and the discharge of its enzymes into the blood stream (particularly when the exocrine duct system was obstructed). However, amylases can be demonstrated in leukocytes,[92] the intestinal wall,[58] and other sites. During a number of extra-pancreatic disorders, the serum amylase level may be increased.[19] Attempts to fractionate tissue amylase into specific components, depending upon the site of origination, have been reported.[182]

The blood of normal persons contains a slight, but persistent level of amylase, and this can be shown to vary with the administration of drugs which affect carbohydrate metabolism.[35] One of the routes of amylase excretion is the urinary system. Thus amylase values in the blood are elevated during renal insufficiency even if pancreatic disease is not present,[101] and the level is slightly depressed during a diuresis.[98]

The occurrence of amylase in the intestine, and its elevation during intestinal infarction,[116,19] suggest that part of the blood amylase increase is due to translocation of the enzyme from the gut to the blood stream. There appears reluctance to accept such a concept in the literature, and numerous experiments have been devised in an attempt to prove that the elevated amylase is due solely to increased discharge of the pancreatic enzyme.[19] The problem can not be resolved definitively until a method is devised for distinguishing between the amylases from different sources.

### Lipase

The pancreas produces a lipase, and blood lipase levels are increased during pancreatic disease.[108] However, the intestine also produces a lipolytic enzyme,[58,173] although this may show species differences.[3] In man, it occasionally appears as if the intestine can produce a lipase of physiologic importance; following complete pancreatectomy, a number of individuals have gotten along well with only moderate reduction of fat intake (suggesting the release of a fat splitting enzyme into the intestine from non-pancreatic sources). The stomach has also been shown to produce a lipase, but this probably has no activity at the low pH values prevailing in gastric juice. What contributions non-pancreatic lipases make to blood levels of the enzyme is unknown. An antilipase is also produced within the body and can be isolated from the urine; the source of the antilipase is uncertain.[108]

### Trypsin

Based on the specificity of trypsin for a synthetic substrate ($\alpha$-benzoyl-L-arginine amide hydrochloride), a test has recently been introduced for the estimation of serum trypsin.[109] In a small series of cases, the procedure was concluded to be more reliable than serum amylase or lipase studies in detecting disorders of the pancreas. Blood levels of trypsin could be detected in normal subjects, suggesting that the enzyme continually entered the blood stream. While the test is 'specific' in the sense that pepsin and chymotrypsin will not attack the substrate, there have been no studies as to the action of the numerous intestinal peptidases on the substrate.

**Pepsinogen**

The urine contains a proteolytic enzyme that disappears after total gastrectomy, and is assumed to arise from the stomach (hence its name, uropepsin). The urinary enzyme is diminished or absent in pernicious anemia, may be elevated in cases of duodenal ulcer, and decreases after partial gastrectomy.[76] The precursor of uropepsin has been assumed to be pepsinogen (the proenzyme of gastric pepsin). Following the intravenous injection of pepsinogen, the uropepsin level increases,[103] indicating that the above assumption may be well taken.

The difficulty of collecting a quantitative 24 hour urine specimen has lead to the investigation of pepsinogen in the blood stream.[76] In man, the plasma level of pepsinogen remains quite constant throughout the day, and is not affected by meals, adrenocotricotrophic hormone or cortisone. Vagal stimulation increases the output of gastric acid, mucous and pepsin. It is thus not surprising that vagotomy is followed by a diminution of plasma pepsinogen.

A low plasma pepsinogen level in cases of gastric carcinoma suggests that the tumors interfere with the production of pepsinogen; this is consistent with the finding that in animals, gastric tumors do not manufacture pepsin or rennin.[62] Hirschowitz has summarized the extensive literature on pepsinogen up to 1957.[74]

A number of important observations and inferences have been made concerning pepsinogen.

1. The blood always contains a small amount of the enzyme, suggesting that its release into the vascular tree occurs normally.
2. When gastric production of pepsin falls, blood pepsinogen decreases; this is highly suggestive that a definite fraction of the pepsinogen produced finds its way into the blood stream, rather than an abnormal shifting of the enzyme in disease states.
3. Plasma pepsinogen levels vary with the ethnic origin of the subject,[30] suggesting genetic factors in its production or release.
4. Total excretion of uropepsin varies between different age

groups and between males and females.[68] Thus a blanket statement as to a 'normal' value is meaningless, but must be interpreted in terms of the status of the individual.

### Alkaline Phosphatase

The intestine contains alkaline phosphatase (s) which are more active than in other organs.[61] From three principal observations, we may infer that part of these enzymes reach the blood stream.

1. Following feeding in rats, the blood alkaline phosphatase level increases in proportion to nutrient uptake. This has been related to the passage of the enzyme from the mucosa to the lymphatic system, and thence to the blood stream.[45]

2. During protein starvation, intraluminal enzymes and blood alkaline phosphatase fall together, and during refeeding, both rise together.[31,134]

3. Using immunochemical methods, Schlamowitz[163] demonstrated that one fourth of the alkaline phosphatase in the blood stream was identical with that from the intestine.

Because of the presence of alkaline phosphatase in the intestine, its response to absorption by entering the blood stream and ready analysis from that location, a potentially useful tool is available for evaluating intestinal function. For precision, what is needed is a means of differentiating intestinal alkaline phosphatase from that of other sources. Inhibition studies have not been successful, but antibodies to intestinal phosphatase have been prepared.[131,132,133,163]

A few observations in the literature suggest that alkaline phosphatase of the gut is sensitive to adrenal steroids. Adrenal stimulation in the chick embryo is accompanied by an increased concentration of intestinal alkaline phosphatase.[82] Corticosteroids increase alkaline phosphatase activity in the duodenum of young rats, while decreasing the absorption of antibodies.[67] In adult animals, the steroids perhaps also enhance activity of this enzyme.[81] The response of serum alkaline phosphatase to steroids[90] (or at least the intestinal contribution to the enzyme) may provide investigative leads in testing intestinal function.

### Acid Phosphatase

Acid phosphatase is present in many cells of the alimentary mucosa.[58,168] The enzyme persists during gastric or intestinal carcinoma.[58,162]

Blood acid phosphatase has multiple components, including contributions from leukocytes,[92] erythrocytes,[1] and the prostate.[44] In the study of acid phosphatases, specific inhibitors have been of much use. Thus, cupric ions and formaldehyde inhibit the enzyme in leukocytes,[1] and L-tartaric acid inhibits acid phosphatase from the prostate.[44] Following L-tartaric acid inhibition, significant acid phosphatase activity is still present in the serum. What fraction of this, if any, is from the intestinal tract is unknown. It would be of interest to follow the serum 'non-inhibited' acid phosphatase during absorption, to observe if there were an increase (in parallel with the rise in alkaline phosphatase).

## Transaminase

Normally, small amounts of glutamic-oxaloacetic acid transaminase can be demonstrated in the blood (as can other transaminases). During experimental small bowel infarction in dogs, the blood level of the enzyme increases.[129]

The relationship of transaminating and deaminating enzymes to the intestine is far more intimate than this. In normal persons, active transaminating systems are present[110,169] in the intestine, as well as enzymes which deaminate glutamine.[170] Certain individuals with celiac disease demonstrate an abnormal increase in plasma glutamine following gluten feeding,[5] suggesting that a deaminating or transaminating mechanism is defective in the gut wall in at least one type of malabsorptive disease.

Adrenal steroids have been recognized to provide at least partial relief from some forms of celiac disease. The recent demonstrations that corticoids enhance transamination processes,[127] opens the possibility that they may be acting on such a system when administered to patients with malabsorptive disorders. The sensitivity of intestinal transaminases to adrenal steroids has been partially investigated.[169]

Whether the release of transaminases from the gut during infarction[129] impairs absorption is unknown. A number of adults who suffered from small bowel anoxia (caused by vessel obstruction due to thrombosis or embolization), were observed to develop a malabsorption syndrome following recuperation.[137,138] (This condition is accompanied, at least experimentally, by the release of intestinal transaminases into the blood stream.)

### Other Translocated Alimentary Enzymes

The enzyme-rich alimentary canal discharges a number of its products into the blood stream. It is probable that a multitude of enzymes, in addition to those already studied, pass from the gastrointestinal system into the circulation. Of particular interest would be blood studies of carboxypeptidases, aminopeptidases, prolidases, and other relatively specific ferments produced by the alimentary mucosa and its appendages. Intensive investigation of enzymes translocated from the gastrointestinal tract may provide a means of quantitating alimentary function, a task that is difficult to accomplish at present. Recently it has been shown that the intestine is a possible source of the proteins which form serum lipoproteins.[180] This, if confirmed, would be another example of protein synthesis in the gut and subsequent translocation of the finished product. There is also a recent report on the synthesis of proteins by the small intestine and their subsequent delivery into the portal circuit.[181]

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# 7

# ENZYMES AND DIGESTIVE PROCESS

PASSAGE OF nutrients from the gut to the body must be preceded by their breakdown into forms suitable for absorption. This process is termed digestion, but no single word is adequate to convey the complexity, specificity and coordination of multiple functions which occur at the same time or sequentially. The prime factors in digestion are enzymes. These biocatalytic molecules permit the necessary reactions to be carried out at the body temperature within short periods of time.

By no means, however, do enzymes tell the entire story of digestion. Electrolytes of the gut secretions are not passive bystanders, but rather are essential for the functioning of various ferments (such as the amylases [60]) . In addition, the mechanical action of the gut is also contributory, as it breaks food materials into smaller particles. When the size of the bolus is reduced, a larger surface area becomes available to serve as the enzyme-substrate interface (this is of particular importance in the cleavage of lipids) . Other ancillary processes (such as the emulsifying action of bile salts) also contribute to the rapidity and success of digestion.

By means of intestinal intubation or drainage from a fistula, an almost clear, viscous fluid can be collected. For want of a better name, and perhaps because of lack of information concerning its composition, the fluid has been termed the "succus entericus." As commonly obtained, this material is a liberal admixture of intestinal secretions, bile, pancreatic juice, and gastric fluid. Rarely can the succus entericus be considered, as usually prepared, to be solely derived from the small gut. Actual digestion takes place in the presence of this medium, formed by the influx of multiple components. Our knowledge of the action of digestive enzymes, in each other's presence, is still incomplete and much work remains to be done before the spatial and temporal sequences of digestion are elucidated. Theories of the origin, translocation, inhibition and fate of the digestive enzymes have been presented in the chapter on Production and Translocation of Gastrointestinal Enzymes and Proteins.

## DIGESTIVE ENZYMES

The number of enzymes involved in the digestive mechanism is large. Fortunately, from the viewpoint of classification, they can be grouped in four headings.

1. Carbohydrases.
2. Esterases.
3. Proteases.
4. Others (as phosphorylases).

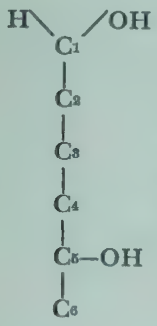
### Carbohydrases

Carbohydrases are enzymes which catalyze the hydrolysis of carbohydrates (simple glycosides, oligosaccharides, polysaccharides, and carbohydrates in complex combinations). Examples of these enzymes are:

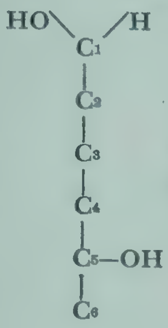
$\alpha$—amylase (pytalin) of saliva
$\alpha$—amylase of pancreas
? amylase of small intestine

Maltase
Sucrase } of small intestine
Lactase

Amylases from salivary and pancreatic juices act primarily on oligo- and polysaccharides which have the groups attached to the asymmetric carbon atom in the alpha configuration. Example:



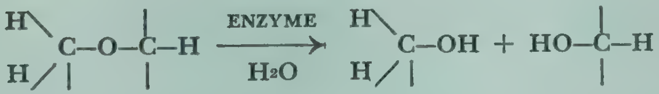
The hydroxyl group of $C_1$ is on the same side as that of $C_5$ This is called the alpha ($\alpha$) configuration.



The hydroxyl group of $C_1$ is on the side opposite to that of $C_5$. This is called the beta ($\beta$) configuration.

Polymers, such as amylose, amylopectin, and glycogen, are formed by carbohydrates being attached, one to another, by means of alpha linkages going principally from $C_1$ of one molecule to $C_4$ or $C_6$ of adjoining molecules. Such bonds are referred to as $\alpha$-1,4 and $\alpha$-1-6. The $\alpha$-1,4 linkages of oligo- and polysaccharides are readily cleaved by salivary and pancreatic amylase (although these two enzymes rarely liberate free glucose). Certain polysaccharides with the $\beta$-bond are split by human amylases (likely after mutarotation). Cellulose with $\beta$-links is not altered in the human gut, except by the action of bacterial enzymes. The occurrence of such bacteria in the gut of ruminant animals allows them to gain nutrition from a diet consisting largely of cellulose fibers.[1] The indigestibility of cellulose in man is shown by the passage of vegetable fibers in the stool. Cellulose, and a number of pentose polysaccharides (also with $\beta$-bonds), serve no other purpose in man than to act as bulk; indeed, they are often used as ingredients in 'bulk laxatives.' Normally, about 300 mg of indigestible fibers are eaten per 100 calories.[58] If $\beta$-splitting cellulases instead of $\alpha$-amylases were present in the human gut, cellulose would be 'food.'

Amylose is a polysaccharide with a linear structure, composed of glucose attached to adjoining glucose molecules by means of $\alpha$-1,4 glucosidic bonds. During digestion, the chain is repeatedly split.



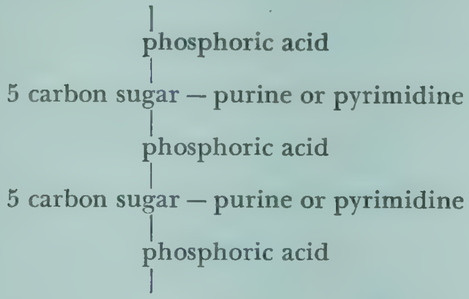
$$\mathrm{H_2\underset{|}{C}{-}O{-}\overset{|}{\underset{|}{C}}{-}H} \xrightarrow[\mathrm{H_2O}]{\text{ENZYME}} \mathrm{H_2\underset{|}{C}{-}OH} + \mathrm{HO{-}\overset{|}{\underset{|}{C}}{-}H}$$

Each of the split products probably serves as a substrate for pancreatic $\alpha$-amylase until the disaccharide maltose is produced. Maltose is not readily cleaved by this enzyme; indeed, it may inhibit further $\alpha$-amylase activity. The final product of amylose degradation, by pancreatic $\alpha$-amylase in vitro, contains primarily maltose with small amounts of other items (however in vivo, hydrolysis may not proceed this far). The presence of enzymes which possess the ability to split long chain carbohydrate polymers, such as amylose, may be estimated by the quantity of reducing sugar produced or by the loss of the polymer's ability to react with iodine to form a blue color. 'Amylase' in the blood stream is usually quantitated by the amount of reducing sugar liberated from a polysaccharide,[50] or by the cleavage of an artificial substrate.[51]

The amylases from salivary juice (ptyalin)[52] and pancreatic fluid (amylopsin)[3] have been crystallized and their $\alpha$-amylolytic activity demonstrated. The optimum pH of both enzymes is near 7. The amylases require the presence of specific ions in order to be effective.[60] Salivary amylase needs chloride ions, but is still active if chloride is replaced by bromide, iodide or nitrate. The amylases are depolymerizing enzymes (cleaving glucose-glucose polymers and similar molecules composed of other carbohydrates); their kinetics have been described in terms of enzyme-polymer system.[4] If the assumption is made that other enzymes necessary for starch digestion are present in adequate amounts in the gut, then starch degradation depends upon the quantity of amylase present; the oral feeding of starch, followed by determinations of blood glucose, has been proposed as an indication of amylase activity.[5] Starch granules normally occur in the stool, and they have little absolute diagnostic value.[55] An amylase inhibitor occurs in wheat starch,[74] but the significance of this substance in vivo is uncertain.

Amylopectin and glycogen differ from amylose in that they possess, in addition to the $\alpha$-1,4 bonds, branchings associated with $\alpha$-1,6 linkages. Cleavage, by $\alpha$-amylase, of the $\alpha$-1,4 sites in amylopectin and glycogen results in a variety of linear and branched oligosaccharides of from 2 to about 10 glucose units. The $\alpha$-1,6 bonds are not split by $\alpha$-amylase, but are cleaved by an intestinal oligo-1,6-glucosidase.[61] Such an enzyme has been demonstrated in the gut of germ-free rats,[2] indicating its existence independent of bacterial activity. Complete hydrolysis of starch to glucose is thus accomplished by the enzymes amylase, oligo-1,6-glucosidase, and finally maltase. The disaccharides maltose, sucrose, and lactose are split by intestinal 'disaccharases' (although the latter two disaccharides contain $\beta$-bonds). Maltase, sucrase, and a weak lactase[63] do not appear to enter the intestinal lumen, but may be acting at the surface of luminal cells.

It will be recalled that a nucleic acid can be represented as follows.

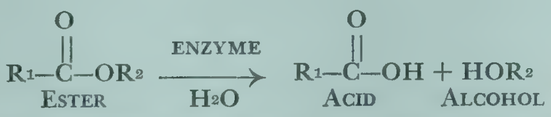


phosphoric acid
5 carbon sugar – purine or pyrimidine
phosphoric acid
5 carbon sugar – purine or pyrimidine
phosphoric acid

A ribonuclease (for ribonucleic acids) and a deoxyribonuclease (for deoxyribonucleic acids), primarily from the pancreas, depolymerize the polymers to nucleotides (units containing phosphoric acid–5 carbon sugar–purine or pyrimidine). From this point on, the steps taken by the intestine are uncertain. A nucleotidase (such as a 5'-nucleotidase) may split the nucleotide to a nucleoside (5 carbon sugar- purine or pyrimidine). However, free base can appear, suggesting further cleavage of the nucleoside, and possible functioning of a phosphorylase rather than a nucleosidase.[6,64]

**Esterases**

Esterases are enzymes which attack the ester linkages of acids and alcohols; the typical reaction may be schematized as follows.

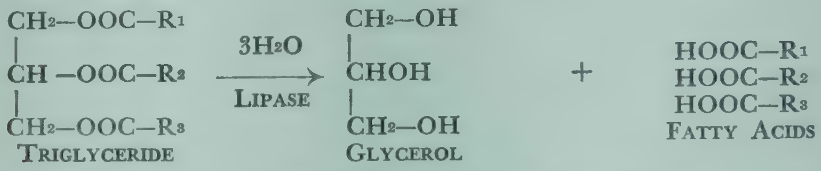


$$\underset{\text{Ester}}{R_1\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}OR_2} \xrightarrow[H_2O]{\text{ENZYME}} \underset{\text{Acid}}{R_1\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}OH} + \underset{\text{Alcohol}}{HOR_2}$$

Esterases of the gastrointestinal tract are divided into two classes: the non-specific esterases (little studied and of unknown significance in human digestion) and specific esterases which carry out highly specialized tasks. The commonest specialized esterases are:

Gastric lipase
Pancreatic lipase
Lipase of small gut[59]
Phosphatases (acid and alkaline)
Nucleases (ribonuclease, deoxyribonuclease)
Nucleotidases
? (Nucleosidases)
Lecithinase[79]

The major part of dietary lipids occur as triglycerides (glycerol linked to three fatty acids). A portion of the triglycerides are hydrolyzed in the gastrointestinal tract to glycerol and fatty acids by special esterases termed lipases.



$$\begin{array}{l} CH_2{-}OOC{-}R_1 \\ | \\ CH{-}OOC{-}R_2 \\ | \\ CH_2{-}OOC{-}R_3 \\ \text{TRIGLYCERIDE} \end{array} \xrightarrow[\text{LIPASE}]{3H_2O} \begin{array}{l} CH_2{-}OH \\ | \\ CHOH \\ | \\ CH_2{-}OH \\ \text{GLYCEROL} \end{array} + \begin{array}{l} HOOC{-}R_1 \\ HOOC{-}R_2 \\ HOOC{-}R_3 \\ \text{FATTY ACIDS} \end{array}$$

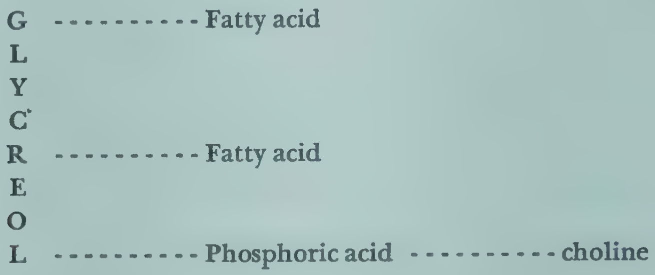
In the stomach, the acidity of the medium would appear to set the stage for lipid digestion. However, no appreciable gastric splitting of lipids can be demonstrated; perhaps the pH is not sufficiently low, the temperature not high enough, the retention period too short. A lipase can sometimes be demonstrated in gastric fluid. Its optimum pH is close to neutrality and activity at acid pH values is very slight or absent. Hence, even if the enzyme were a gastric secretion, it would have little action in vivo. The suggestion has been put forth that gastric lipase plays a role in lipid digestion during infancy, since gastric acidity is lower than in adulthood, and milk lipids occur highly emulsified (both conditions favoring action of the enzyme).

Pancreatic lipase probably arises in the form of a precursor which is activated by an unknown mechanism. This lipase is group-specific and hydrolyzes a number of esters. Lipase activity is greatest when substrates containing fatty acids of intermediate chain length (such as lauric and myristic acids, which possess 12 and 14 carbon atoms respectively) are used. Estimation of the activity of lipase in vivo has been attempted by two procedures. 1. Intestinal contents can be removed by intubation and assayed for enzyme content by the hydrolysis of a known substrate.[7] In such situations, it may be difficult to determine if the enzyme arose from the pancreas or from the small gut. 2. Fats labeled with radioactive tags (such as triolein-$I^{131}$), when fed orally, are split to glycerol and labeled fatty acids which are then absorbed (some partially split products may also be taken up). In any event, little of the radioactive tag appears in the feces. Hence, the use of triolein-$I^{131}$ can be regarded as a functional test of the ability of the gut to hydrolyze and absorb lipids.[8] In order to distinguish between digestive and absorptive errors, oleic acid-$I^{131}$ (which does not depend on cleavage to simpler products for its absorption) may be employed.[9] The tests assume that $I^{131}$ follows the carrier fat, and this appears to be a valid assumption.[62]

The splitting of lipids involves three materials: the lipid, water, and lipolytic enzymes (which are water soluble). Since lipids are not soluble in water, lipolysis must occur at the lipid-water interface. The rate of reaction is approximately proportional to the area of the interface. Progressively smaller droplets, with a larger surface area, are a result of the process termed emulsification. Glycocholic and taurocholic acids from bile, at the pH of the intestine, act as detergents or emulsifying agents. These agents separate the lipid droplets in the aqueous medium. Such action, plus the mechanical activity of the gut, results in the dispersion of small lipid droplets throughout the luminal fluid. Soaps and monoglycerides, resulting from the digestion of fats, also act as detergents and supplement the activity of bile. The presence of bile may not be entirely essential for lipid digestion and absorption. Following bile exclusion, lipids in the stool are still partly in hydrolyzed form. Fat intolerance observed in some patients with biliary disease suggests that handling of lipids is retarded in the absence of bile, but there may be wide individual differences. Characterization of the lipase reported to be present in the succus entericus has not been described.

We have already mentioned that nucleic acids are depolymerized by a deoxyribonuclease and a ribonuclease from the pancreas, and perhaps by somewhat similar enzymes from the gut wall. The resultant nucleotides (phosphoric acid—5 carbon sugar—purine or pyrimidine) are acted upon by phosphatases or nucleotidases to split off the phosphoric acid (a 5'-nucleotidase has been identified in the gut). This step can be viewed as the result of a specialized esterase (splitting the bond between phosphoric acid and the alcoholic hydroxyl on ribose or deoxyribose). The next step, (questionably performed by a nucleosidase), is also an esterase function in the broadest sense (or a carbohydrase function), and the sugar and nitrogenous base are separated. Deoxyribonuclease of pancreatic origin requires the presence of divalent magnesium ions for its action. Both deoxyribonuclease and ribonuclease have been crystallized [10,11] and studied as to chemical composition.[12,13] Ribonuclease has also been obtained as a radioactive crystalline enzyme following the incubation of tissue slices with $C^{14}O_2$.[14] Because of the importance of deoxyribonuclease (often abbreviated DNA'ase), a number of methods have been evolved for its estimation.[15,16,17]

Lecithins are complex lipids (a type of phospholipid) in which a choline group is attached to the phosphoric acid moiety of the molecule:

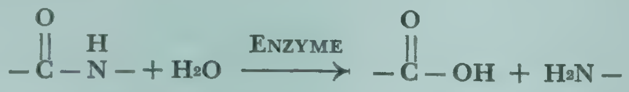


If the phosphoric acid-choline group is attached to the middle carbon of the glycerol, the resulting compound is termed a $\beta$-lecithin. Intestinal mucosa is said to contain a lecithinase, capable of degrading lecithin, but its action was, until recently,[79] unclear. If the phosphoric acid group were split off, the molecule remaining would be a simple diglyceride. The venom from certain snakes, and perhaps from bees, has been noted to contain enzymes which remove the fatty acid from the $\beta$-carbon atom of lecithin. The resulting substance, termed a lysolecithin, has toxic properties. The production of lysolecithins by intestinal bacteria has not been clearly demonstrated. There has also been described an enzyme, found in P. notatum and commercial pancreatin, which catalyzes the migration of the fatty acid acyl group (R. CO.) of a lysolecithin from the middle (beta) to the outside (alpha) position on glycerol.[18] The action of this enzyme in the gut, if any, is unknown.

Numerous phosphatases can be found in the mucosal wall,[66] and weak activity may be demonstrated in the alimentary fluid (perhaps due to the sloughing of cells). Phosphatases split organic phosphorus compounds, and perhaps also function in their resynthesis during absorption. Their role is discussed in other sections.

**Proteases**

Proteases are enzymes which cleave the -C-N- bonds of proteins and peptides:



$$-\overset{\overset{O}{\|}}{C}-\overset{H}{N}- + H_2O \xrightarrow{\text{Enzyme}} -\overset{\overset{O}{\|}}{C}-OH + H_2N-$$

They are commonly subclassified as proteinases (endopeptidases) or peptidases (exopeptidases). Enzymes of the latter group are usually inactive against proteins, but attack peptides and peptide derivatives which possess free amino or carboxyl groups (see review by Smith[78]).

Proteinases hydrolyze "interior" peptide bonds of proteins; occasionally they also attack this bond in simple peptides, esters and their derivatives. Principal examples of the proteinases are:

- Pepsin
- Trypsin
- Chymotrypsin
- Rennin (animals, ? from stomach of infants)

Within the intestinal tract, pepsin and other proteinases do not cleave proteins all the way to simple amino acids, but rather produce peptides. The term "erepsin" was introduced to designate the enzyme responsible for the final liberation of amino acids from the peptides formed (from proteins) by proteinase action. The concept of one enzyme doing the entire final splitting process has proved to be too simple. A multiplicity of enzymes are involved, and these are termed, logically, peptidases. The peptidases may be divided into two subgroups, depending on whether they attack the amino or carboxyl end of the peptide. Principal peptidases are:

- Carboxypeptidase (pancreas)
- Aminopeptidases (from many locations)
- Aminotripeptidase
- Leucine aminopeptidase
- Glycylglycine peptidase
- Prolidase

The peptidases or exopeptidases as they are sometimes called, are usually incapable of attacking protein 'interior' peptide bonds. Their action is often limited to cleaving peptide bonds which adjoin terminal free alpha-amino ($H_2N$-) or terminal free carboxyl (HOOC-) groups. Hence, the exopeptidases are limited in their workings to relatively simple structures.

The statement is usually made that coagulated proteins are more readily digestible than the native form; hence, one of the supposed rationales for cooking food. It might be argued that denaturation unfolds protein molecules, and hence exposes formerly interior bonds to enzyme action. Experiments to test such an hypothesis appear to be few in number. Trypsin does not attack native hemoglobin, but digests denatured hemoglobin under identical conditions.[19] Tryptic digestion of egg albumin has been reported to increase as the protein is denatured.[20] Skim milk powders, prepared at various temperatures, are absorbed at equal rates,[21] probably indicating that the materials are equally readily digested. However, overcooking may impair the biologic availability of some amino acids.[56] In certain cases, the digestion and absorption of proteins occurs with such facility, that the disappearance of nitrogen from the gut lumen is equal to that which occurs when amino acids are fed (showing that the steps in digestion proceed rapidly[22]).

The proteinases are the best characterized of the digestive enzymes, even though there is still much to be uncovered concerning their activity. We will briefly discuss each of the major proteinases in turn.

### Pepsin

The breakdown of food proteins begins in the stomach. Beaumont clearly demonstrated this action in 1833, while studying digestion by means of a gastric fistula. Schwann introduced the name pepsin to describe the meat digesting factor of gastric juice.

Isolated gastric mucosa does not display peptic activity. However, an inactive precursor (pepsinogen) has been separated and crystallized.[23] Pepsinogen is probably synthesized by chief cells of the gastric glands. The molecular weight of pepsinogen, its isoelectric point, stability in alkaline solutions, and inactivity toward proteins, establishes it as an entity distinct from pepsin. Pepsinogen is a 'proenzyme' or 'zymogen' and is activated to pepsin,[24] probably by splitting off part of the molecule. The conversion process proceeds at a pH under 6 and is autocatalytic (that is, pepsin itself speeds the action). One view is that pepsin splits several peptide bonds in pepsinogen, unmasking the active enzyme core. There is evidence that the polypeptide produced inhibits further pepsin production.

Pepsin has been crystallized,[25] and may be analyzed by its action in producing tyrosine from a hemoglobin substrate,[26] and by other techniques.[57] Pepsin and the exopeptidases, unlike the endopeptidases, do not require free -COOH or -$NH_2$ groups in the terminal positions of the substrate. Peptic activity in the blood stream has been mentioned in a previous chapter. The optimum pH for pepsin varies with the substrate, but is generally about pH 2. The functioning of an enzyme under such acid conditions is remarkable. The activity of pepsin falls off as the pH is raised; irreversible changes occur at about pH 6. Hydrochloric acid, secreted by gastric parietal cells, is approximately pH 1.5 and is well suited to providing an environment favorable for pepsin. In achlorhydria, the probability is that pepsin has little digestive power. During the treatment of peptic ulcer, administration of antacids not only neutralizes HCl, but also indirectly protects the crater by altering the pH of the lumen and creating conditions far from the optimum for peptic digestion. Pepsin is inactivated by trypsin and the alkaline medium of the duodenum; its action probably ceases soon after the material leaves the stomach.

The specificity of pepsin for protein substrates is not the same as that exhibited by other enzymes. A discussion of the specificity requirements of the proteinases is given in the text of White *et al.*[27] The action displayed by certain proteinases in splitting artificially prepared substrates, as well as ester linkages, has formed the starting point for assay of the enzymes.

### Rennin

This enzyme appears to be absent from adult human gastric juice, although claims have been made that it is present in the stomach secretions of infants. Rennin is prepared commercially from calf stomach and may be crystallized free from pepsin.[28] In common with many other proteinases, rennin causes the coagulation of casein in the presence of divalent calcium ions. The enzyme is of interest, since it forms the basis of a number of coagulated milk products, and has also been identified in the splitting of phosphoric acid from phosphoproteins[29] (phosphamidase action). An additional proteolytic enzyme, with a pH optimum near 3.2 has been isolated from the gastric contents and named gastricsin.[65]

**Trypsin**

When acid chyme from the stomach enters the duodenum, it meets alkaline secretions of the pancreas and duodenal wall. Pepsin is rendered inactive and protein digestion would cease if other proteolytic enzymes were not present. The pancreas secretes two enzymes (trypsin and chymotrypsin) which continue the breakdown of protein.

A proteolytically inactive precursor of trypsin, trypsinogen, can be obtained in crystalline form from the pancreas.[75] Trypsin is generated from this 'proenzyme' in neutral solution; trypsin catalyzes the reaction which is therefore termed 'autocatalytic.' Pancreatic extracts also yield a basic peptide which combines with trypsin forming an inactive compound.[30] As mentioned previously, other trypsin inhibitors are known, including ones in soybeans, eggs, and human and bovine colostrum[31] (which may function to prevent digestion of antibodies in colostrum). A trypsin inhibitor is present in blood,[32,76] and approximately 1 milliliter of serum inactivates 1 milligram of trypsin. Within the gut lumen, production of trypsin from its precursor is favored by enterokinase, a secretion of the intestinal wall.[68,69,75] The purification of enterokinase,[80] and study of its properties,[81] revealed it to be an enzyme which functioned by splitting a peptide from trypsinogen.

Trypsin is assayed by its action on an artificial substrate,[33] and the kinetics of tryptic digestion have been investigated.[39] Clinically, estimation is often made by the ability of duodenal fluid or stool specimens to digest gelatin in a tube, or on an exposed X-ray photograph (bacterial enzymes may yield false positive results). Diminished quantities of trypsin in the duodenum result either from pancreatic disease (as cystic fibrosis) or from obstruction of the inflow tract (such as a stone in the common duct or neoplasms of the ampulla of Vater). Trypsin inhibits spontaneous movements of the isolated rabbit intestine (an action shared by chymotrypsin),[34] but whether this is operative in vivo is unknown. The enzyme also has effects on the response of the isolated intestine to different medications.

The presence of small quantities of trypsin in the blood stream, and elevation of its concentration during pancreatic disease (as measured by activity against an artificial substrate), is discussed in the chapter on Production and Translocation of Gastrointestinal Enzymes and Proteins.

**Chymotrypsin**

This enzyme can be functionally distinguished from trypsin since it promotes the clotting of milk, but not of blood, while trypsin has the opposite effects. Both trypsin and chymotrypsin hydrolyze CTpNP (N-carbobenzoxy-L-tyrosine p-nitrophenyl ester),[35] demonstrating that the enzymes possess some esterase activity in addition to their role as proteinases.

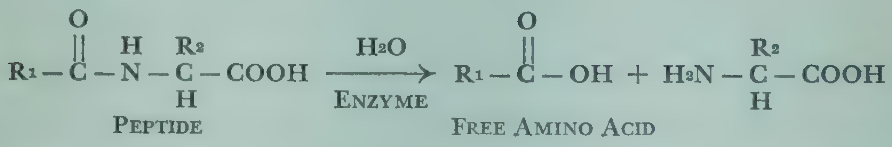
An inactive precursor, chymotrysinogen, can be isolated from the pancreas.[67,72] Conversion of this proenzyme to the active product is promoted by trypsin, but not by chymotrypsin (and hence is not autocatalytic). A mildly alkaline pH favors the reaction, and the overall process perhaps again involves the splitting off of a peptide fragment. After activation, a number of chymotrypsin-like molecules may be isolated, each having a different molecular weight.[36,73] This has been interpreted as meaning that the essential enzyme function is located in a specific core of the molecule, and that the 'nonessential' segments may be split off without adversely influencing activity.

Chymotrypsin stability and activity are enhanced by calcium.[37] The enzyme, in both its esterase and proteinase actions, is inhibited by diisopropylfluorophosphate (DFP- which also serves as an inhibitor of cholinesterase [38]). Carboxypeptidase is not altered by DFP, hence carboxypeptidase can be estimated in the presence of chymotrypsin after the addition of DFP. The remarkable thing about DFP inactivation is that such a small molecule can render the huge protein-enzyme molecule ineffective. This suggests that the "active core" is rather small (histidine and serine have been implicated); there is some evidence, however, that chymotrypsin acts as though it effectively had more than one active site.[53] Chymotrypsin is quantitated by its action on artificial substrates,[33,35] and its kinetics have been studied. Westheimer has reported on the substrate specificity and mechanism of action of chymotrypsin,[40] and Cunningham has put forth a scheme for the action of hydrolytic enzymes in general.[41]

Proteins entering the digestive canal are subjected to the action of pepsin, trypsin, and chymotrypsin. The majority of proteins are split by these proteinases, but the fibrous keratins from a notable exception. The keratins are insoluble materials which are apparently protected by disulfide bonds (-S-S-), from both solution and enzyme action.

**Carboxypeptidase** [70,71]

The pancreas liberates, through its ducts, an enzyme precursor (procarboxypeptidase) which is converted to carboxypeptidase by the action of trypsin (and perhaps other enzymes [42]). Carboxypeptidase has been crystallized,[43,44] and the enzyme from bovine sources contains zinc.[45] Carboxypeptidase, as its name suggests, cleaves the peptide bond adjacent to a free carboxyl group; an amino acid is liberated.



$$R_1-\overset{\overset{O}{\|}}{C}-\overset{H}{N}-\underset{H}{\overset{R_2}{C}}-COOH \xrightarrow[\text{Enzyme}]{H_2O} R_1-\overset{\overset{O}{\|}}{C}-OH + H_2N-\underset{H}{\overset{R_2}{C}}-COOH$$

Under special circumstances, pancreatic carboxypeptidase catalyzes the hydrolysis of proteins by splitting off amino acids successively from the terminal carboxyl end; this has proven of value in determining the amino acid sequence of proteins. It is essential that the carboxyl group be free; masking of -COOH inhibits carboxypeptidase action. Carboxypeptidase is only active against L-amino acids (and not the synthetic D-isomers). The preferred amino acids in the terminal position of the peptide, for carboxypeptidase activity, appear to be the 'aromatic' amino acids, phenylalanine and tyrosine.

Carboxypeptidase can be assayed by its activity in hydrolyzing carbobenzoxyglyclyl-L-phenylalanine. The enzyme thus exhibits a limited esterase activity. The effect appears to be restricted to compounds which have a steric arrangement analagous to acceptable peptide substrates. There are several known inhibitors of carboxypeptidase, including acids of the type R.COOH (as phenylproprionic acid; R is similar to part of a terminal amino acid residue). The enzyme is stable from pH 6 to pH 10, but may be altered by drying.

### Aminopeptidase

The endopeptidases and carboxypeptidase leave unaffected the $\alpha$-amino terminus of peptide chains. The task of sequentially hydrolyzing amino acids from this end of the peptide molecule is handled by a multitude of enzymes termed aminopeptidases. Knowledge of these enzymes is fragmentary, and none of them have yet been crystallized. There is uncertainty as to their substrate specificity. Two facts have emerged from studies of the aminopeptidases. (1) There is probably more than one point of attachment between enzyme and substrate. (2) Metal ions play a role in the functioning of many aminopeptidases (Table 6).

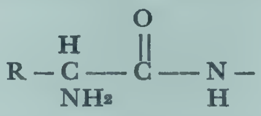
TABLE 6

CHARACTERISTICS OF SOME PEPTIDASES

| *Enzyme* | *Metal Required for Activity* | *Comment* |
|---|---|---|
| Leucine aminopeptidase | Mn++[47] Mg++ | The amino acid must be unsubstituted. The enzyme acts on both di- and polypeptides. |
| Glycylglycine dipeptidase | Co++[48] | Free carboxyl and amino groups both essential for activity. Does not act on the tripeptide, glycylglycylglycine. |
| Tripeptidase | None known[46,49] | Activity limited to the alpha-amino end of the peptide. |
| Prolidase | ? Mn++[49] | Splits the imino group of proline. |

von Euler and Josephson[77] demonstrated that glycylglycine which had been acylated (benzoylglycylglycine) was not attacked by glycylglycine dipeptidase. The conclusion was that for successful activity, the enzyme had to bind the substrate at two sites (the amino group, plus a peptide linkage being hydrolyzed). Still later, it was found that a free carboxyl group was also required for the enzyme to be active. Hence, the enzyme may bind the substrate at three points (polyaffinity theory). Polyaffinity is also a useful concept in viewing the spatial specificity of other enzymes.

Even in minute concentrations ($10^{-5}$ molar), metals activate various aminopeptidases. Smith[47] suggested that the metal and substrate formed an intermediate compound (metal complex) which was then attacked by the enzyme. The side chains of the amino acids influence the rate of hydrolysis by aminopeptidases. In the peptide:



$$R-\underset{NH_2}{\overset{H}{C}}-\overset{\overset{O}{\|}}{C}-\underset{H}{N}-$$

the hydrolytic rate is stated to increase as R increases ($H < CH_3 < C_2H_5$; the side chain may thus play a role in binding enzyme to substrate). This relative specificity is augmented by an absolute specificity for L-amino acids. Some aminopeptidases show activity only against dipeptides, while others attack tripeptides as well. The net result of the action of the endopeptidases and exopeptidases is the reduction of complex proteins to amino acids.

### Other Enzymes

The role played by other enzymes (phosphorylases, dehydrogenases, oxidases, decarboxylases, isomerases, condensing enzymes, transferases) in the digestive process is uncertain, and will not be discussed here. Materials foreign to the normal diet may occasionally be split by enzymes in the gut lumen, in the intestinal wall, blood stream, or liver. Three enzymes of uncertain significance in digestion are $\alpha$-1, 3-glucosidase,[82] dextranase[83] and cholesterol esterase of liver, intestine and pancreas.[84]

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# 8

# GASTROINTESTINAL HORMONES

CONTROL OF enzymes, fluids, and motility, of necessity, requires a complex and closely regulated mechanism. The existence of systems which integrate digestion by means of a 'feed-back' of information from the gut, have been proposed, but little studied. Thus, the protein secreted by the gut in response to eating varies with the makeup of ingested proteins,[1] and the composition of pancreatic secretions depends upon the composition and degree of fluidity of the diet. Certain mechanisms have been recognized, but the picture is still far from complete.

1. Dietary proteins influence the enzymes secreted.[2,3] Part of this may be due to stimulation of particular chemoreceptors and hormones, and part undoubtedly results from the great metabolic activity of the pancreas[4] and its dependence upon an adequate supply of amino acids for enzyme synthesis.

2. Chemoreceptors have been tentatively identified in the gastrointestinal canal,[5,6] although it is not entirely clear if they are sensitive to specific stimuli or to large classes of compounds. In addition, receptors to chemical stimuli are present in blood vessels leaving the visceral region.

3. The extrinsic nervous supply of the gastrointestinal canal modifies activities and probably also affects the blood supply to and from the region.

4. The alimentary canal itself secretes hormones which regulate its activities. This is a magnificent servomechanism, but has received little attention. The gastrointestinal hormones are integrated with nervous mechanisms to produce a unified scheme of action. As an example, Dragstedt and coworkers[7] describe the regulation of gastric secretion as follows. Gastric secretion is stimulated by re-

flexes via the vagus nerve as well as by a hormone from the antrum of the stomach (gastrin). When intragastric contents reach pH 3, gastrin is no longer released, and further acid secretion is inhibited. Acid food passes to the duodenum, and this is the stimulus for the liberation of another hormone, pancreatic secretin. This latter substance has an inhibitory action on gastric secretion, while stimulating the pancreas. The net effect is to prevent excessively acid intraluminal materials from coming in contact with the stomach or duodenum.

Some aspects of the nervous and neurohumoral regulation of intestinal activity are presented in summary volumes by Youmans[8] and Babkin.[9] In this chapter, a brief outline of gastrointestinal hormones will be given; we will not discuss serotonin (see chapter on Carcinoid Tumors), the effect of exogenous hormones on the gut, or the action of substance P (a polypeptide which causes intestinal contraction, but whose activity in vivo is unknown [10]).

Hormones produced by the gastrointestinal canal are generally of protein composition. After synthesis, they may travel a circuitous hemic path, only to end up by exerting their effect at another site in the alimentary tract. An unusual aspect of these hormones is that they regulate the function of exocrine glands (rather than endocrine ones); however, some of the enzymes produced under their stimulus may pass into the blood stream in small amounts. Since the gastrointestinal system is both origin and effector site for its hormones, extirpation experiments are nearly impossible to perform. Many investigations designed to elucidate the function of alimentary hormones have been poorly controlled, and in some cases the very existence of individual hormones is open to question. The literature on the subject, up to 1950, has been quite thoroughly summarized by Grossman,[11] and his paper may be consulted for details. Grossman concluded that secretin and cholecystokinin were the only two gastrointestinal hormones which could be conceded to be established from the view points of endogenous physiologic release and presence in tissue extracts. Evidence for the existence of gastrin, pancreozymin and enterogastrone was considered strong. The presence of duocrinin, enterocrinin and villikinin, had yet to be confirmed by other laboratories, while urogastrone and anthelone could be demonstrated but had no apparent physiologic function. In the interval since Grossman's paper, no works have appeared to cause an alteration of these statements.

## SECRETIN

This was the first gastrointestinal hormone to be demonstrated;[12] recent summaries of information concerning secretin have appeared.[24,27] Secretin is liberated from the alimentary mucosa in response to chemical agents and stimulates the release of pancreatic fluid. The material can be obtained relatively free of histamine and other substances which cause vasodilatation; Legge and coworkers[13] have discussed purification of secretin by physical methods. The hormone is produced in the upper intestine and perhaps also in the stomach; the stimulus for release is hydrochloric acid, but the efficacy of other substances has not been determined. Secretin is assayed by biologic methods,[14] and has enjoyed clinical use in stimulating pancreatic secretion during tests of exocrine function of that organ. Interestingly, secretin also stimulates the rate of hepatic bile secretion; its action is hydrocholeretic[15] (that is, it increases the volume of flow per unit time but does not stimulate the outpouring of bile pigments or salts). Secretin is most effective when given intravenously, less so by other parenteral routes, and ineffective orally (but slightly active when given per rectum). Upon incubation with plasma, secretin becomes inactivated; an enzymatic action has been postulated as being responsible for this effect. Secretin is deficient in hypophysectomized rats,[25] but the effect of other hormones on secretin production is unknown. Vagotomy results in a reduction in secretin, but not in pancreozymin.[28]

## CHOLECYSTOKININ

A hormonal factor produced in the mucosa of the upper small intestine, released in response to surface contact with various chemicals, and capable of eliciting contraction of the gallbladder, is termed cholecystokinin.[16] Appropriate chemical stimuli for release of the hormone are hydrochloric acid, fats and fatty acid, and protein split products. Cholecystokinin is assayed by biologic means, and is distinct from substance P (which is also a smooth muscle contracting agent). The urine of man contains a substance which contracts the gall bladder (and hence has been termed urocholecystokinin [26]).

## GASTRIN

Mechanical and chemical agents call forth a hormone from the gastric mucosa which stimulates gastric glands to release hydrochloric acid (but apparently does not stimulate pepsin liberation). In analogy to secretin, the hormone has been called gastric secretin or gastrin. Proof of existence of the hormonal factor was shown by the use of autotransplanted stomach pouches, which were demonstrated to secrete acid in response to a meal in the main stomach.[17] Gastrin is principally produced in the pyloric region, and the action of the hormone is reviewed by Dragstedt and coworkers.[7] There has been much discussion in the literature as to the possible relationship of gastrin to histamine release, with histamine being the 'final common pathway' in hydrochloric acid secretion. This point is still far from settled.

## PANCREOZYMIN

Harper and Raper[18] were able to demonstrate the presence of a second hormone (in addition to secretin) which evoked pancreatic secretion. The substance was produced by the intestinal mucosa in response to materials in the lumen. The difference between secretin and pancreozymin is that the latter calls forth pancreatic fluid rich in enzymes (and resembles vagal stimulation in this respect), while secretin elicits a watery flow that has few enzymes. Atropine perhaps prevents the release of pancreozymin. Biologic assay is used to test the potency of pancreozymin preparations.

## ENTEROGASTRONE

Mucosa of the upper small intestine produces a hormone (enterogastrone) when fats, soaps or sugars are present in the lumen, which inhibits gastric motor activity and acid secretion.[19] Assay of the hormone is by biologic means; effects similar to those of the hormone can be elicited by bacterial pyrogens, and hence rigid control of the preparation is necessary. The delay in gastric emptying has been used as a measure of the activity of the hormone, but other factors may contribute to this effect.

## DUOCRININ, ENTEROCRININ, VILLIKININ

These three substances are of uncertain existence and importance. Duocrinin[11] is a hormone stated to be released by the intestinal mucosa which is effective in causing secretion from Brunner's glands. Enterocrinin is supposedly a hormonal agent from the intestinal mucosa which regulates the secretion of the jejunum and ileum,[20] while villikinin is said to be secreted by the intestinal mucosa to stimulate movement of the villi.[21]

## UROGASTRONE, ANTHELONE

Urogastrone is a substance which can be obtained from the urine of animals and man. When given parenterally, urogastrone inhibits gastric production of hydrochloric acid.[22] The substance apparently exists, but no physiologic role has been demonstrated. Anthelone is obtained from intestinal mucosa or urine, and prolongs the survival of dogs subjected to the Mann-Williamson operation (which usually leads to death by the production of peptic ulceration[23]). Again, there is no demonstrated function in intact animals.

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## 9

# ABSORPTION*

THE COMPLEX process of digestion may be viewed as a step preliminary to the uptake of nutrients from the alimentary lumen. Subsequent movement of ions and organic molecules from the lumen to the body proper is called absorption. This transport of materials a fraction of a centimeter is the vital step in intestinal function; without absorption all other activities of the gut are meaningless. It must be recognized at the outset that multiple physical and chemical factors are involved in absorption. Thus, such diverse elements as intraluminal pressure, concentration gradients and osmotic pressure, time of contact, diffusion, and electrical activity of the cell, have been noted to be of importance in the absorption of various materials. On the other hand, it would be an unwarranted oversimplification to say that four or five factors recognized at present are the whole story of absorption.[115] Indeed, the more we know about the uptake of materials from the lumen, the more certain it becomes that most of the operations involve an expenditure of energy and can be explained only in terms of the metabolism of cell membranes.[1] This does not mean we are to throw up our hands in a concession to the 'vitalists.' In the period since 1945, there have evolved both experiments and theories to treat the topic of 'active absorption' across a membrane (that is, in the face of an electrochemical gradient [162,163,164]). We will only state here that such transport systems generally invoke the concept of a 'carrier' to convey the material under consideration across the membrane, as well as an energy supplying system which separates substrate from carrier, thus reconstituting the carrier for further action.

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*Defects of absorption are discussed in the chapter on Malabsorption.

'Absorption' is such a simple word that we tend to regard the activities it represents as simple. Of course, this is not so. As a first approximation, we may say that absorption (especially that proceeding through alimentary luminal cells) consists of three substages.

1. Passage of a material from lumen to cell. This, itself, may be a complex event, involving carriers and an energy supplying source.

2. Processing of the metabolite by the cell. A prime example is the conversion of carotene to vitamin A in the gut wall.

3. Release of the processed substance to the blood stream or lymphatics. In addition to our noting that absorption is more than one step, we can list a few generalities concerning these events; most of the statements have more value in a negative sense (what absorption is not) than in a positive one.

a. Absorption is often quite 'class specific.' That is, there is no guarantee that the processes involved in carbohydrate uptake are the same as those concerned with lipid uptake.

b. The gut wall can not be viewed as a uniform organ when it comes to absorption. Site specificities are often shown (such as the favored uptake of iron in the duodenum and jejunum).

c. Transport across the gut wall may be possible for one material but not for a closely related substance. Thus, ferrous iron is absorbed, whereas ferric iron is not.[2] L-amino acids are absorbed, but the D-forms are absorbed at a slower rate, if at all[3,4,165,166,167] (there are species differences). Such observations suggest that one or more steps in the absorptive process are exquisitely specific as far as steric effects are concerned.

d. The absorption of any dietary constituent normally takes place from a complex medium which contains other compounds. The results of this process may differ from absorption when no other substance is present. Thus, carbohydrates may vie with each other for absorption, and the same is true for amino acids.[5,6,89] Further, there may be 'cross interference' between groups, with certain compounds reducing the absorption of others.[7,169,23]

In some cases, the added agent has been shown to combine with the substrate, producing a form which was not suitable for absorption. Well known examples of this phenomenon are: phytic acid binding of calcium[8] and iron,[178] egg white complexing of biotin,[9] and the possible reaction of beta-sitosterol with cholesterol.[10] As an added complication, bacterial destruction of an ingested test material will also result in spuriously depressed absorption, such as thiaminase destroying thiamine.

e. Observations indicate that uptake of certain substances can be enhanced by the presence of other compounds (suggesting the use of the added material as a 'cofactor' in some way). In many instances, the observations are inconclusive, although the following have been noted: various carbohydrates increase calcium absorption from the gut, a number of amino acids (as well as lactose) increase calcium and strontium absorption in rats, d-sorbitol enhances vitamin $B_{12}$ uptake in man, d-sorbitol increases iron absorption,[11] and glucosamine may have some effect in enhancing tetracycline absorption[12] (although reports are conflicting).

Absorption may be affected by a number of different events in vitro, and in vivo. The thyroid hormone, for example, has an effect on absorption which, at least in part, is due to its general action on cellular metabolism. Much attention has also been directed to the relationship of motility to absorption. Instances of increased motility (as in certain cases of diarrhea) may be accompanied by diminished absorption, but this effect is difficult to separate from the decreased time of contact between substrate and surface. It is probable that a finite, but short, interval is required for absorption, and that this necessary "contact time" varies with the type of nutrient and other factors.

Available surface area[120] is also a key factor in determining the extent of absorption. This is true whether passive diffusion or active transport is taking place. Most substances appear to be absorbed over a wide area of intestine, with a large functional reserve. Surgical removal of part of the intestine can result in malabsorption[121, 170,132] by diminishing the absorptive area; the requisite surface appears to vary between individuals. Surgical extirpation of part of the digestive tract makes clear cut experiments difficult, since it may also remove accessory factors necessary for digestion (such as intrinsic factor produced in the stomach). Functional recovery from small bowel resection may be associated with intestinal hypertrophy in some cases,[170] but not all.[181]

From this brief background, we proceed to examine the absorption of specific substances. Mention must be made, however, that many of the difficulties encountered in understanding absorption are due to the fact that no single test determines the extent of uptake of a material from the gut lumen. Quantitating the disappearance of a metabolite from the lumen, or its entrance into luminal cells, is difficult, if not impossible, in vivo. Hence, other lines of experimentation have been used, and resultant data are often difficult to interpret. This subject is discussed more fully later in connection with tests of intestinal absorption.

## CARBOHYDRATES

With the exception of certain individuals with congenital enzymatic errors (see section on Development), carbohydrates are absorbed only in the form of monosaccharides. Uptake of these sugars can not be explained entirely on the basis of simple physical factors such as diffusion and osmotic gradients. This is borne out by four observations.

1. Carbohydrate absorption from the gut lumen is not the same for all molecular species, but varies in the following increasing order (suggesting some selectivity by the gut wall): arabinose, xylose, mannose, fructose, glucose, galactose.[13] (When introduced into the peritoneal cavity, all of these sugars are absorbed at the same slow rate.)

2. The movement of carbohydrates across the gut wall is independent of concentration after a 'critical' concentration is reached.[36] Furthur, the most rapidly absorbed carbohydrates may interfere with each other's uptake, pointing to competition for one or more transport systems. (There is recent, unconfirmed, work that more than one system is operating since D-glucose was absorbed after the intestine was presented with a 'saturating' load of 1-deoxy-D-glucose.[183])

3. The newborn chick absorbs a variety of carbohydrates at the same rate. A few days later however, certain sugars are preferentially absorbed, as though an 'active system' had matured.[15]

4. Rats starved for 48 hours absorb less fructose than those denied food for only 24 hours.[35] Interestingly, an increase in absorptive abilities following starvation has also been reported.[186]

The nature of the active transport system has not been elucidated. Schemes involving phosphorylation and subsequent dephosphorylation have been proposed,[17,47] primarily because of the following reasons.

1. During glucose absorption, the concentration of certain organic phosphates in the gut is noted to increase,[16,20,171] and phosphorylated substances can be recovered.[20,22]

2. Poisoning the intestinal mucosa with iodoacetate[17] or phlorizin[18] (both known to inhibit phosphorylation) blocks the rapid absorption of galactose and glucose, but not the slow diffusion of the 'passively' taken up pentoses.

By no means however, can the phosphorylation theory be considered to be firmly established. Each of the above observations can be countered by serious discrepancies. While it is true that iodoacetate and phlorizin block phosphorylation, they are also protoplasmic poisons and affect other systems as well (iodoacetate inhibits enzymes containing the -SH group, and phlorizin interferes with at least one other enzyme[172]). The occurrence of phosphorylated substances in the intestinal wall tells us little as to whether they are essential for absorption or are simply 'storage' or 'intermediate' forms. Indeed, recent work has disclosed that the majority of absorbed glucose does not pass through the mucosal pool of glucose-6-phosphate.[184] Also damaging to the phosphorylation theory are experiments using glucose derivatives. Thus 3-methylglucose is not readily phosphorylated by the intestinal mucosa,[173] but is absorbed.[174] A deoxysugar is one in which an alcoholic -OH has been replaced by -H. Removal of the -OH group means that phosphorylation can not occur at that point. However, the various deoxysugars studied thus far (1-deoxy-,[183] 2-deoxy-,[174] and others) are absorbed. The phosphorylation theory of carbohydrate absorption is thus at a low ebb. Its ultimate fate depends upon furthur experimental data and the advancement of alternate hypotheses.

There are other aspects of carbohydrate absorption which are poorly understood at present, such as why fructose is converted to glucose during absorption.[168,154] This process involves schism of the fructose molecules.[187] Amino acids and glucose administered orally at the same time result in depression of the absorption curves (as measured by blood levels) of both materials;[23] a single simple mechanism does not readily explain this observation. Glucose absorption also varies with the portion of the gut investigated,[24] suggesting that the absorptive mechanism is not equally active over the entire length of intestine. After a critical intraluminal concentration is reached, glucose absorption is linear with time ("Cori's Law") [14,36] as though the absorptive capacity was saturated. Hence the amount of glucose which can be absorbed from the gut is limited.[36]

Absorption of polysaccharide breakdown products occurs at a rapid rate, even when the environmental temperature is altered,[25] indicating that the time spent in digestion is minimal. Conflicting reports as to whether carbohydrate absorption diminishes with time[13,26] may tentatively be related to varying periods of gastric emptying.[27,28]

Carbohydrate absorption is linked to other little understood factors, such as the presence of hormones and electrolytes. Uptake of carbohydrates from the gut lumen is inhibited by hypophysectomy, adrenalectomy, and sham operations.[29] Insulin and thyroid hormone also influence the movement of sugars from lumen to mucosa (see chapter on Hormones). In the frog, potassium increases the rate of intestinal absorption of carbohydrates, while calcium salts have an opposite effect.[30]

## LIPIDS

Despite intensive study for many years, the exact nature of the process responsible for fat absorption has not been defined. Indeed, there is still debate as to the type of lipid product taken from the lumen into mucosal cells. Part of the difficulty has been in constructing experiments which would clearly indicate when lipid absorption had occurred. As a starting point, we will review a number of procedures which have been employed, and will note the difficulties encountered.[31]

### 1. Lipid recovery from stool

Following a test meal, it would appear logical to assume that lipids appearing in the stool came from those ingested. However, this is not true. Such a conclusion is backed by a number of observations.

a. On a fat free diet, lipids continue to appear in the stool. Lipids must thus pass from the blood stream or lymphatics into the lumen, and this is confirmed by chemical analysis.

b. Lipids are continually entering the lumen from the mucosal surface, and the rate of entry is increased by radiation damage.[32] Normally, only a small amount of secreted lipid leaves in the stool; it is subject to reabsorption before the colon is reached.

c. Following oral administration of radioactive fat, lipid recovered from the stool reveals a product with a lower specific activity (radioactivity/weight of fat) than that administered.[33,34] This again suggests dilution of fat by mixing or exchanging with 'endogenous' lipids. All of these observations indicate that simple balance studies may reveal little as to the mechanism of fat absorption, unless account is taken of the secretion of lipids, and lipid 'recycling.'

Lipids may be chemically recovered from stool or intestinal fluid.[37,38] One technique is to administer a fat tagged with a radioactive isotope ($C^{14}$ and $I^{131}$ are commonly employed) [39,40] and then detect excreted radioactivity. As we have mentioned, this value may be of little quantitative significance unless account is taken of lipid dilution by endogenous stores.

**2. Appearance of ingested lipid in blood and tissues**

Absorption of lipids is necessarily followed by their appearance in blood and tissues. However, relying on blood levels to indicate the extent of absorption is fraught with dangers, as shown by the following data.

a. Although lipid may be taken from the gut lumen, it does not necessarily have to appear immediately in the blood stream. Indeed, in the case of vitamin A, temporary storage of the vitamin in mucosal cells has been tentatively demonstrated.[41] Hence, an initial delay in the appearance of test material in the blood is not necessarily indicative of slow absorption, but may represent distribution in a storage area.

b. The liver is interposed between the intestine and the general circulation. Lipids absorbed via the portal circuit may be stored or altered by the hepatic system. Studies are needed on lipid absorption in the absence of the liver. Such investigations are technically feasible, since the liver can be temporarily excluded in certain experimental animals.[42]

c. It is frequently forgotten that the blood level of a lipid is the result of the interplay of absorption and utilization. Blood lipids are but a fraction of the total body lipid pool, and interchange readily takes place. Peripheral clearance may thus rapidly alter the blood level. Hence studies such as chylomicron counts, serum turbidity, and total lipid values after a fat meal[43] must be viewed with caution, despite reports to the contrary. When interpreted in comparative terms, such studies have clinical validity and importance.

### 3. Other parameters

The disappearance of lipids from the gut lumen has been studied. This is a valuable parameter but too complex for routine clinical use at the present time. Similarly, studies of lipid utilization, when coupled with blood studies, are of value; again however, the methodologies are too involved for everyday use. It is thus apparent that there are not absolute criteria available for studying lipid absorption. This is one of the major reasons for the lack of detailed information regarding lipid breakdown and absorption.

While carbohydrates are primarily absorbed in the upper portions of the small intestine, this is not necessarily true of lipids in all species. By use of fat stains, fat absorption can be histologically demonstrated at all levels of the small gut. Experiments which selectively removed parts of the intestine have shown that in both the dog[44] and the rat,[45] the distal portion of the small gut was primarily charged with lipid uptake.

Rather than go into chronologic details of the evolution of present concepts of lipid absorption, we will present the data now available. Bile salts (salts of glycocholic and taurocholic acids) act as emulsifying agents by arranging themselves so that lipophilic groups (as $CH_3$-,-$CH_2$-) face the lipid and hydrophilic groups (COOH, -OH) are orientated toward the water phase.[46] Such an arrangement separates the lipid globules, increases the surface area, and allows pancreatic lipase to perform its task. It is now apparent that, within the gut lumen, lipolysis does not go to completion. That is, not every molecule of fat is degraded to glycerol and fatty acids;[52] complete hydrolysis does not appear to be essential for uptake, as monoglycerides can be absorbed.[182]

Pflüger had formulated the 'lipolysis theory' which stated that fats were completely hydrolyzed in the gut, absorbed as digestion products, and subsequently reconstituted to triglycerides in the gut wall. (Evidence has recently been presented that, in vitro, fatty acids placed at the mucosa partially appear in the form of triglycerides at the serosal side.[188]) The reconstituted triglycerides were viewed as entering lymphatics, while bile salts returned to the lumen via the liver and biliary secretions. The lipolysis theory was primarily based on observations recorded by Munk and Rosenstein (1891). These workers studied a patient with a lymph fistula draining from the thigh. After feeding either triglycerides, fatty acids, or esters of fatty acids, the majority of the ingested products could be isolated from lymph in the form of neutral fats. Following each fat meal, the previously clear lymph became turbid, due to the presence of multiple small fat droplets. The constant appearance of the droplets and their chemical identification as neutral fats were assumed to be strong evidence for lipolysis in the lumen, reconstitution of fats in the gut wall, and liberation of the end product into the lymphatic stream. The theory was brought to its fullest development by Verzar and McDougall,[47] who claimed that fatty acid absorption was preceded by the formation of complexes between fatty acids and bile salts in the upper intestine.

The lipolysis theory actually presented two distinct concepts: the breakdown of fats prior to absorption, and uptake entirely by the lymphatics. The attractiveness of the theory was the fact that fats are not water soluble, and their degradation prior to absorption seemed logical. An offshoot of the lipolysis concept was the saponification of fats (soap formation) prior to absorption. This latter hypothesis could not be substantiated, but it was not until 1934 that the lipolysis theory was subject to thorough analysis. In that year, Frazer[48,49] advanced the view that fat absorption was a combination of two events—uptake of fat digestion products into the portal circulation, and passage of emulsified particulate fat into the lymph circuit. Recently published photographs of the intestinal villus, taken by means of the electron microscope, strikingly demonstrate the entry of particulate fat droplets into mucosal cells.[189]

Because of the dual nature of absorption in this scheme, it has been termed the 'partition theory.' (Recent evidence of normal lipid absorption despite ligation of the thoracic duct of the rat, lends support to a dual or complex mechanism of lipid uptake.[180])

In the absence of bile, fat absorption is diminished but still occurs,[50] adding weight to the contention that complete splitting of fat to its breakdown products is not essential prior to absorption. Despite the fact that in some experiments most of the administered fats appear in the lymphatic system,[51] there is evidence for the partition theory. For instance, some fats are split to monoglycerides prior to absorption but not into smaller fragments (free fatty acids and glycerol), showing that hydrolysis can be partial but not complete.[52] The real key to the matter may be in the differential partition of fatty acids with carbon chains of different length. Short chain fatty acids are apparently absorbed into the portal circuit, while longer fatty acids (over 10 carbon atoms) pass into the lymphatics. Fatty acids labeled with radiocarbon have provided interesting data along this line. A fatty acid with 16 carbon atoms showed 85 per cent passage into the lymph of rats, while only 70 per cent of a 14 carbon fatty acid entered, and but 15 per cent of a 12 carbon fatty acid.[53] The point has been made that milk is the only common dietary product rich in the shorter chain fatty acids. Until more evidence has been gathered, it would be wise to keep our minds open on the subject of fat absorption, but to tentatively view:

1. Partial hydrolysis as being sufficient for the absorption of some fat from the lumen,

2. Fatty acids being reconstituted to triglycerides in the intestinal wall. (The intestinal mucosa can not convert glycerol to $\alpha$-glycero-phosphate;[179] the exact pathways of reconstitution are still uncertain.)

The transfer of fatty acids from lumen to mucosa can take place against a concentration gradient,[55] and involves an expenditure of energy. Since the mechanism is affected adversely by phlorizin and dinitrophenol, phosphorylating systems have been thought to play an important role in the process. During fat uptake from the lumen, there are changes in the composition of intestinal phospholipid (although the total amount may remain constant). Sinclair[56] hypothesized that phospholipids were important vehicles in the transport and resynthesis of lipids, and later studies employing tagged phosphorus compounds have shown rapid intestinal phospholipid turnover during fat absorption. Much additional work remains to be performed before the role of phospholipids in fat absorption is clarified.

The relationship of bacteria to lipid interconversions and absorption is incompletely known. Cetyl alcohol, the primary alcohol corresponding to palmitic acid, can be isolated from the stool. It is possible that bacteria affect the reduction of the acid to the alcohol. Blomstrand and Rumpf[54] have reported that labeled cetyl alcohol can be converted to palmitic acid by the intestinal mucosa of the rat. Whether this is a true mucosal function or is simply due to bacteria in the wall is unknown. Of great interest is the demonstration that the ether linkage of chimyl alcohol can be ruptured by the small intestine of man and the rat.[190] This is one of the few known examples of ether linkage cleavage in mammals.

During fat absorption, the concentration of alkaline phosphatase has been noted to increase in the blood stream.[57] This has been attributed to a translocation of the enzyme from the gut wall into the lymphatics. Quantitative studies of the phenomenon are not available. Alkaline phosphatase of the gut wall can function in transphosphorylating processes,[58] but the significance of this in the absorptive process has still to be clarified.

Normal lipid absorption appears to be dependent upon an intact stomach. The evidence is incomplete, but the following data are available.

1. Patients who have had a major portion of their stomach removed show multiple nutritional difficulties (such as defective iron uptake, and other absorptive abnormalities leading to weight loss and anemia). In such patients, the absorption of radioactive fat may be shown to be diminished.[59]

2. The gastrectomized dog also undergoes metabolic derangements postoperatively. Amann and Brunchwig[60] found that im-

plantation of a small patch of stomach into the efferent jejunal loop partially reduced the nutritional impairment. They suggested that this might indicate a factor, aside from conventional secretions, required for fat absorption from the gut.

3. After gastrectomy, rats gain weight for 2 to 3 months, and then lose weight until the time of death, 6 to 12 months postoperatively. Neutralized human gastric juice has been reported by Balfour, Higgins, and Woods,[61] to partially prevent this weight loss shown by gastrectomized rats, and to increase the time of their survival. However, no altered fat excretion was observed, by chemical means, in the stool of animals so treated.

The complexity of the changes following gastrectomy[83] (diminished hydrochloric acid, altered transit time, decreased manufacture of intrinsic factor, and perhaps other aberrations) make evaluation of the deranged lipid absorption difficult. Hence it is not possible to state at the present time whether the stomach produces a lipid optimal absorption factor (LOAF) or not. Recently, it has been shown that when radioactive fat is given orally, more is lost in the stool if the patient has been fasting than if a meal has been taken. This suggests that the elevated radioactive fat excretion observed after gastrectomy is primarily due to an inadequate mixing of fat with digestive enzymes and fluids.[145] The stomach may have other relationships to lipid metabolism; oral administration of gastric mucin has a 'plasma clearing effect'[62] (clearing the plasma of lipids) much like heparin, but the relationship of this to lipid absorption is unknown (it may be related to the fact that the alimentary canal produces proteins which can bind serum· chylomicrons [185]).

## STEROIDS

The steroid nucleus is possessed by a number of compounds of major importance in the body economy. Although mammals can synthesize cholesterol and most other steroids from acetate, large quantities of preformed cholesterol are presented to the gut. For instance, an average daily diet contains 0.5 grams of cholesterol, but the value may be over 5 grams when meals rich in cholesterol are chosen.[63] Studies with labeled cholesterol have revealed that 20 to 50 per cent of an oral tracer dose is absorbed[64,65] (varying with the amount ingested and the total lipid intake; there is probably an upper limit to cholesterol absorption of about 2 grams per day[68]). Cholesterol within the gut lumen arises from at least three sources.

1. The major portion consists of cholesterol from the diet, which failed to be absorbed.
2. The gut wall secretes cholesterol.
3. Normal bile contains 8 to 25 mg cholesterol per 100 ml.[66]

Estimation of the intraluminal cholesterol pool is possible by two techniques (a depletion method and a balance method),[67] which give results on the same order of magnitude (about 2 grams in man [73]). This cholesterol pool is in constant flux, with material entering,[74] some being degraded or leaving in the stool, and additional molecules crossing the mucosa and being recycled via resecretion.[68] In addition to other factors, the size of the pool of recirculated cholesterol depends upon the level of fat intake.[82] Cholesterol within the intestinal lumen may undergo the following pathways: absorption and recycling, absorption and degredation, and breakdown by bacteria. Some 87 per cent of cholesterol metabolized in the body goes to bile acids[68] of a number of types.[75] There is some evidence that bile acids interfere with cholesterol absorption.[155] (Bacterial metabolism of cholesterol within the gut is discussed in the chapter on Intestinal Bacteria.)

Cholesterol absorption appears to proceed via the lymphatic route,[69] and the suggestion has been made that cholesterol transferred from lumen to lacteals is esterified.[70] Upon entering the mucosa, cholesterol is diluted into a large pool of free and esterified cholesterol.[146] In the rat, the lower small intestine may be more capable of cholesterol absorption than the upper portion.[71] The tremendous load of cholesterol available for absorption can perhaps best be shown by a hypothetical case (see also discussion of Alimentary Clearance later in this chapter). If 1 gram of cholesterol was ingested and 0.5 grams passed in the stool, we would say that absorption was 50 per cent efficient. However, we must realize that additional cholesterol is entering the gut lumen via secretion from the gut wall (which can also synthesize cholesterol) [72] and from the bile. In reality, then, the mucosa must vie with 1 gram of ingested cholesterol, 0.2 grams from bile, and 2.0 grams from intestinal secretions (a total of 3.2 grams). Of this, all but 0.5 grams are successfully reabsorbed or prevented from leaving; mucosal cell efficiency for cholesterol absorption is then $\frac{3.2-0.5}{3.2}$, or 84 per cent.

Absorption of cholesterol appears to be highly selective in that bacterial reduction products of cholesterol and various plant sterols (phytosterols), are not taken up (although this has been questioned[78]). Certain other steroids, however, are absorbed, and hence can be administered via the oral route. These include:

Digitalis and related compounds (which are also partially secreted by the biliary system)[76]
Adrenal steroids
Vitamin D
Dihydrotachysterol (AT-10)

The plant sterols β-sitosterol and dihydro-β-sitosterol (Figure 2) differ from cholesterol in having an ethyl group on the side chain, and in saturation of the ring in the dihydro compound.[77] These modifications are apparently of enough importance to prevent ready absorption of phytosterol molecules. There is some evidence, however, that labeled β-sitosterol may be partially absorbed, and in this manner interfere with cholesterol uptake.[78] The administration of the phytosterols increases fecal cholesterol excretion, often with a reduction of the blood cholesterol concentration. The mechanism responsible is uncertain; the formation of a nonabsorbable phytosterol-cholesterol complex has been mentioned,[10] as well as competition for absorption.[78,79] It has been reported that β-sitosterol taken orally does not lower the lipid content of bile,[66] suggesting entry of cholesterol into the lumen but an absorptive failure in the presence of phytosterol. A steroid from wool also interferes with cholesterol absorption,[80] by an uncertain mechanism. Another compound, delta-4-cholestenone[81] is absorbed from the intestine and subsequently interferes with the conversion of acetate to cholesterol in the liver. Clinical use of these compounds is still in the developmental stage.

## AMINO ACIDS

Aside from instances of intact protein incorporation (from colostrum) early in the neonatal period of certain animals, and possible uptake of proteins in allergic states, the absorption of proteins can be considered to be almost entirely in the form of amino acids.[176,177] The site of amino acid passage from lumen to the body proper is in the small intestine; unabsorbed amino acids are largely decarboxylated or otherwise acted upon by bacteria in the large

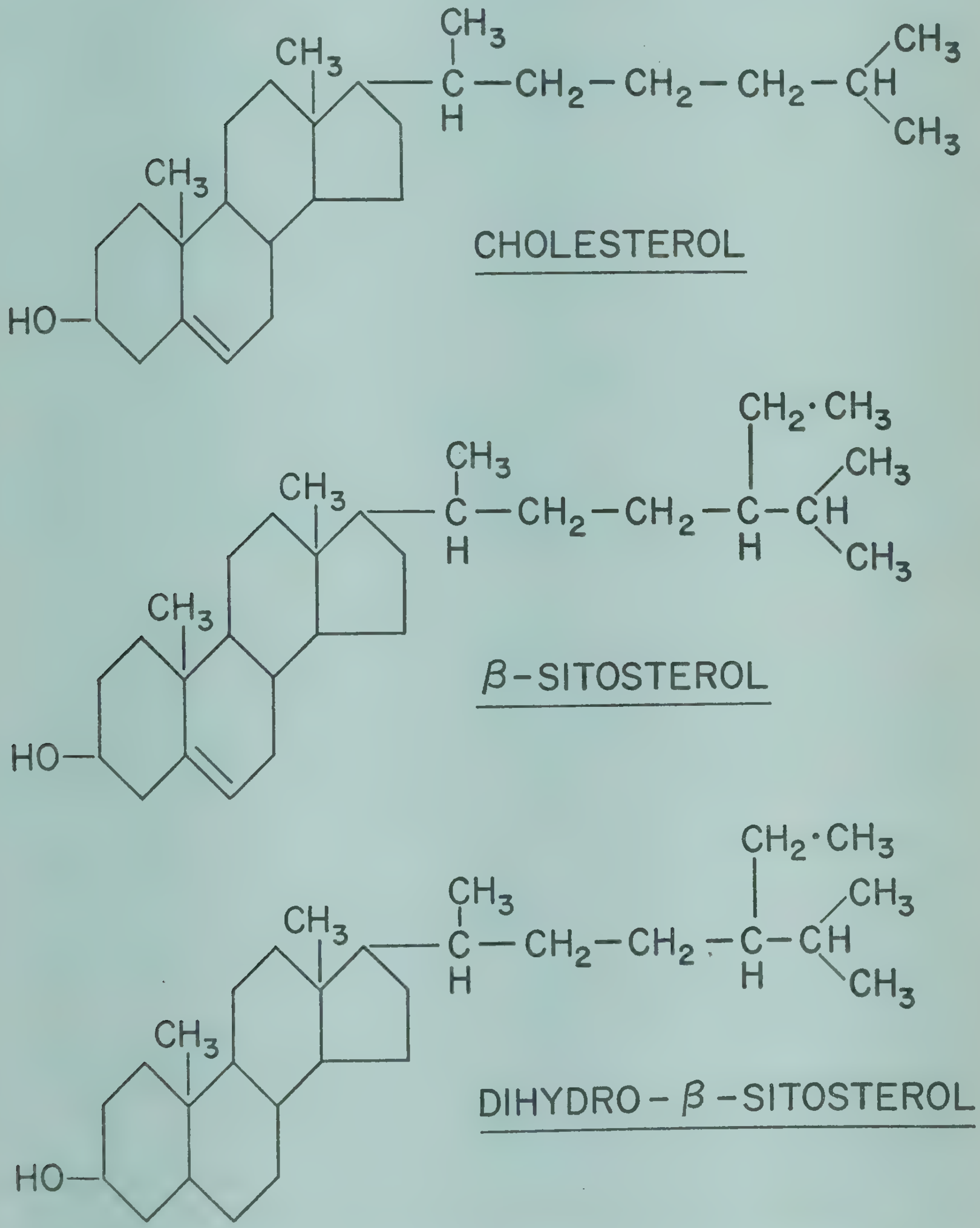
CHOLESTEROL
β-SITOSTEROL
DIHYDRO-β-SITOSTEROL
HO
CH3
CH2·CH3
C
CH
H
CH2

Figure 2. Structural formulae of cholesterol and two phytosterols.

gut. There is perhaps no unique area of the small gut within which amino acid uptake occurs, although in man, absorption may be complete before the ileum is reached. Once through mucosal cells, amino acids primarily appear in the portal circuit; small amounts may be present in lymph channels.[84]

Demonstration that uptake of certain amino acids from the gut was an active process[85] (and not diffusion alone), served as a stimulus for studies attempting to clarify the mechanism involved and its specificity. Data presently available show the following.

1. The mechanism is specific in that some amino acids are taken up against a concentration gradient, whereas others are not[89] (there may indeed be more than one mechanism). Further, the rate of absorption of even the actively transported amino acids may vary from one molecular species to another.[86]

2. There is stereospecificity, and naturally occurring L-amino acids may be actively taken up while the D-analogues are unaffected,[87] or taken up only slowly.[88] (This recently has been brought into question.[21]) Beta-alanine and serine are taken up more rapidly than isoserine, suggesting that absorption decreases as the amino group is displaced from the carboxyl radical.[90]

3. Active transport of amino acids is inhibited by anaerobic conditions, by cyanide, and by dinitrophenol.[3,91] The process, however, is not readily blocked by phlorizin;[91] thus, although phosphate in the gut may change during amino acid uptake,[92] the process must differ from that operating during carbohydrate absorption (which is inhibited by phlorizin). Chlortetracycline, an antibiotic known to depress phosphorylation, partially inhibits histidine absorption.[19]

4. Some of the amino acids which are actively transported, mutually inhibit the uptake of one another.[89,93] This effect may depend upon the concentration of the amino acids.

5. The rate of absorption of amino acids depends upon the concentration in the lumen, until an upper limit is reached.[91] Whether this represents a saturation of the absorbing mechanism is unknown.[3] Mention should also be made that in vivo, there are compensatory mechanisms (via the outpouring of proteins from mucosa to lumen) which tend to maintain the intraluminal amino

acid concentration at a fairly constant level[94] during absorption.

6. Using in vitro techniques, the amino acid appearing on the serosal side is usually chemically identical with that absorbed. However, transamination may occur in the intestine, and glutamic and aspartic acids appear to undergo this process during their absorption.[87] Further, the intestine may transaminate amino acids present in the blood which passes through its substance.[95]

Amino acid absorption from the intestine is thus primarily an energy requiring process, which is highly selective. The specific steps involved have yet to be elucidated. The amino acid absorbed may be chemically identical with that placed at the mucosal surface, or may be a transamination product in some cases. There is evidence that amino acid uptake is dependent upon the presence of pyridoxine[161] and that carrier intermediates are formed.

## ABSORPTION OF NUCLEIC ACIDS

High molecular weight nucleic acids are degraded to simpler materials before passing from the lumen to mucosal cells. In vitro experiments[96,175] suggest that nucleosides (nitrogenous base-pentose) are the units which undergo uptake in the gut. Other evidence is quite scanty at present.

## WATER AND ELECTROLYTES

Water can be absorbed at all levels of the intestinal canal. Further, water transport may not entirely parallel solute movement, indicating a somewhat selective mechanism.[98,157] A review of some current ideas on the subject can be found in a paper by Visscher.[97] The total flux of water from mucosa to lumen, and vice versa, is tremendous (involving about 8 liters of secretion plus 1 liter of ingested fluid). The pituitary pressor hormone increases the absorption of water from the intestine in certain isotonic preparations,[99] but whether this is of significance in vivo is uncertain. Mercurials inhibit intestinal water absorption as well as electrolyte uptake,[100] but this too is of unknown importance in intact preparations. Water absorption has been reported to be increased by elevated intraluminal pressure;[101,102] this may partially explain the great absorption of water from the colon (where the pressure is high, but it neglects active factors which regulate water movement). The common electrolytes generally appear to move readily from lumen to mucosa, and vice versa, with probable active uptake of sodium.[147] Ion movement against a concentration gradient involves an energy expenditure which is perhaps supplied by high energy phosphate bonds.[158] Additional aspects of this movement are discussed in the chapters on Isotonicity and the Dumping Syndrome, and Malabsorption.

## MEDICATIONS

The great majority of medications in use are absorbed from the intestinal lumen. Mechanisms involved in the absorption of drugs probably vary, depending upon the chemical nature of the material, physical factors,[104] and the pH.[103] Not only are medications absorbed from the stomach and the gut, they are also secreted into the lumen,[105,76] and the secretion may not necessarily be a simple affair.[105] Determination of the per cent of medication absorbed, using an inert indicator, has been discussed.[103] The absorption of medications may also be affected by other factors in the diet.[148] The absorption of antibiotics divides these medications into two large classes: those which pass through the mucosa and those which do not.[106] Streptomycin is the most important antibiotic not readily absorbed,[107] and along with neomycin and a number of other similar substances, is used in reducing the bacterial population of the gut (antibiotics, however, may interfere with intestinal enzymes and absorption [19,149,150].)

## OTHER SUBSTANCES

Uptake of vitamins from the gut lumen is discussed in the chapter on Vitamins. Information on the absorption of a number of common materials is given below.

### Cobalt

As a constituent of vitamin $B_{12}$, absorption depends upon intrinsic factor. Inorganic cobalt may be absorbed, as well as cobalt-organic moieties.[108]

### Magnesium

Diets containing 0.25 to 0.3 grams per day keep an adult in magnesium balance. Of the ingested magnesium, 60 to over 80 per cent is excreted in the stool,[109,110] presumably due to poor absorption. In severe diarrhea, almost none of the dietary magnesium is absorbed.[111] Proteins may have some effect in enhancing magnesium uptake.[112] Magnesium sulfate is one of the commonly employed cathartics, and draws fluid into the gut lumen.

**Calcium**

Average diets contain 0.8 to 1.0 grams of calcium per day. The majority is passed in the stool (about 80 per cent [113]). Calcium absorption is a complex affair and depends upon vitamin D, perhaps upon the blood level, and on proteins in the diet.[112] In steatorrhea, calcium forms soaps with nonabsorbed fatty acids, and this further interferes with calcium uptake. Phytic acid binds calcium[8] and has been used clinically to reduce calcium absorption. Aluminum compounds may also complex calcium, and this can assume importance during the treatment of peptic ulcer with aluminum hydroxide. Calcium uptake is perhaps favored by more acid pH values and by lactose, but the in vivo data is difficult to interpret. Malm[138] has presented an extensive review of calcium absorption and the presence of calcium in gut secretions.

**Phosphorus**

The ratio of calcium to phosphorus in the diet is about 2.3. Their exact relationship in absorption is uncertain. Some dietary phosphorus may be entrapped in nonsoluble fibers and hence unavailable for absorption. About 30 per cent of ingested phosphorus appears in the stool[159] and some 'endogenous' phosphorus also enters the feces.[160]

**Fluorine**

Daily intake of fluorine is about 0.25 to 0.35 mg per day; an additional 0.5 mg may be taken up from fluoridated water. Certain fluorine compounds are absorbed at a greater rate than the free ion.[114]

The uptake of iron, copper, and iodine are dealt with in other chapters.

## ABSORPTION FROM THE RECTUM AND SIGMOID

The lower intestinal segment has been used for the administration of certain fluids and medications, particularly in the following instances:

1. Patients who are comatose, have gastrointestinal obstruction or other lesions which prevent swallowing;

2. To introduce medications into children when there is resistance to the oral route;

3. Administration of medications such as aminophylline which are rendered less effective by digestive fluids of the upper gut;

4. Instances in which the medication would interfere with gastrointestinal activity if given orally.

The partition of rectally absorbed materials between lymphatics and the blood stream has not been determined. Amino acid absorption from the rectum, when administered in the form of protein, is slight[116] to negligible.[156] Glucose absorption from this area is also minimal.[117] Mercurial diuretics are ineffectively absorbed from the rectum[118] while aminophyllin is taken up well. Water is taken up from the lumen of the rectum and the absorption may be favored by the high intraluminal pressure. Absorption from the rectum is not enhanced by the application of hyaluronidase.[119]

Administration of medications by rectum[122] poses a number of problems.

1. Self-administration may deliver a varying dose unless suppositories are used. The bulk material of the suppository must be nontoxic, pliable, and exert no detrimental effect on absorption. Cocoa butter, glycerinated gelatin and polyethylene glycol[123] have all been used as suppository bases.

2. Active digestive enzymes are not present in the rectum and materials intended for absorption must be given in 'predigested' form.

3. Veins carrying materials away from the rectum generally drain into the portal circuit and hence subject the substance to the action of the liver before reaching the general circulation. Incidentally, it is these veins, without valves and tortuous in their course, which contribute to rectal varices.

4. There are considerations of isotonicity during rectal administration of fluids or medications. Concentrated solutions tend to draw out water, while hypotonic solutions drain away electrolytes (and enemas have been shown to seriously deplete the body of potassium—see chapter on Potassium Metabolism).

5. The purity of medications given rectally must be strictly established, just as though any other route of administration were to be used. There is a report that paraldehyde (which on standing may decompose with the liberation of acetic acid) when used rectally after a long shelf-life, caused destruction of the bowel mucosa.[124]

## DISINTEGRATION OF MEDICATIONS WITHIN THE INTESTINE

There are two aspects of 'delayed disintegration' medications meriting consideration.

1. Coating materials have been used to prevent a medication from being affected by the gastric juice (and in turn, this also prevents the drug from acting on the gastric mucosa). Such preparations are useful when gastric secretions inactivate the compound administered, or when the drug is irritating to the stomach (such as aspirin).

2. Capsules and other dosage forms are presently available which disintegrate in some regular fashion so as to maintain blood levels of a drug over a period of hours. One method of achieving this is to bind the medication in a resin, from which it is released slowly and nearly independently of pH or enzyme activity.[125,126] With success in the choice of a resin-drug complex, the rate of dissociation of the medicine from the binder is a function of available ions in the gut. Since this quantity is relatively constant, the rate of exchange is also roughly constant. There is stated to be a maximum value to the amount of drug which can be removed from the resin; hence, even if the resin-drug complex stayed in the gut, the dose from one day would not be carried over to the next.

A conventional approach to the problem of rendering medications 'enteric,' and thus bypassing the effect of gastric juice, has been to coat the substance with a material not acted upon by fluid in the stomach, but dissolved by intestinal secretions. Coatings commonly used include:

phenyl salicylate (salol)
keratins
shellac
resins and esters.

The effectiveness of these materials depends, in part, upon strict control over their thickness about the medication; when this is not adhered to, the coating may be ineffective.[127] Certain esters (as cellulose acetate-phthalate) are supposedly resistant to gastric acid, but susceptible to attack by intestinal esterases; hence their use as coatings.

The United States Pharmacopoeia describes an in vitro test for determination of the disintegration time of medications. In vivo, actual disintegration may differ from the test tube method. Evidence has been presented that a reliable estimate of the time of disintegration can be obtained by in vivo roentgenographic studies.[128, 129] A radiopaque material is placed within the coating under study, and its disintegration studied by means of periodic roentgenograms of the abdomen.

Pharmaceutical concerns are offering a number of medications in the form of multiple small coated granules. The thickness of the coating varies from granule to granule and during any time interval it is claimed that a fairly constant number disintegrate, thus releasing the drug for absorption.

## CLINICAL TESTS OF INTESTINAL ABSORPTION

There is no single test which gives a quantitative estimate of the functioning of the intestine. Balance studies and tolerance tests have enjoyed clinical popularity, often without critical appraisal. For instance, if a material were to be used in a tolerance test (oral administration and subsequent sampling of the blood stream to calculate the amount absorbed), the following criteria would have to be fulfilled.

1. Absorption from the gut lumen occurs as soon as the material reaches the duodenum, so that variations in gastric emptying time can be ruled out.

2. Absorption of the material must be independent of bile or accessory factors.

3. Structure of the material must be simple so that it is not further degraded by intestinal enzymes.

4. Absorption must not be inhibited by other factors in the diet or in the intestine (does not chelate).

5. The substance must not be degraded by intestinal bacteria.

6. Prior absorption of the compound or similar materials must not inhibit subsequent uptake.

7. Once absorbed, the material must not be stored or metabolized by mucosal cells, but must be rapidly discharged into pathways leading from the gut.

8. The test compound must pass through the liver unaltered and enter the general circulation independently of hepatic function.

9. The compound can not be recycled into the gut via digestive secretions.

10. The substance can not be lost via the renal apparatus or metabolic inactivation during the course of the experiment.

It is apparent that no substance available fulfills all of these criteria. Indeed, it is perhaps well that this is so, since the uptake of one type of material does not always parallel that of compounds of different chemical constitution. Hence, no single test can provide definitive data as to intestinal absorption. We will discuss here the commoner tolerance tests and their limitations.

### Carbohydrate Tests

1. *Oral Glucose Tolerance Test.* Glucose is taken in a dose of 1 gram per kilogram of body weight, or in a standard 100 gram lot. Blood samples are withdrawn periodically and the glucose content estimated. The blood glucose level is supposedly indicative of the competence of intestinal absorption. The curve is high in diabetes, hyperthyroidism and liver disease, and may show an early rise and then a fall in postgastrectomy patients (see chapter on Isotonicity and the Dumping Syndrome). In addition, a significant portion of the supposedly normal population shows a flattened glucose tolerance test (see chapter on Malabsorption). The oral glucose test can thus not be considered to be a definitive procedure for evaluating intestinal absorptive abilities.

2. *Oral Galactose Tolerance Test.* Upon ingestion of a standard dose of galactose,[130] the concentration increases over a period of time. A 'flat' curve occurs in some cases following intestinal resection, while exaggeration of uptake supposedly occurs in hyperthyroidism. Recently it has been shown that galactose metabolism

in the small intestine, of rat and rabbit, is sensitive to stimulation by certain steroids.[191]

3. *Oral Xylose Tolerance Test.* D-xylose is a five carbon sugar (a pentose). Absorption of xylose from the intestine is perhaps preceded by phosphorylation,[144] although this has been questioned. Once in the blood stream, xylose is excreted by the kidneys with little tubular reabsorption. Hence, the amount of the pentose in the urine is a good approximation of xylose absorption.[131] Urine is collected for 5 hours after ingestion of 25 grams of d-xylose. Normally, 4 to 6 grams are excreted in this period. In malabsorptive states (q.v.), less is absorbed and excreted, and the excretory pattern may not markedly change following therapy. Some clinics follow the blood level of xylose after oral ingestion, but this appears to add little to the information obtained. A complicating factor in use of the test is that d-xylose is not completely inert metabolically. Small (and questionably variable) amounts are metabolized.[192,193]

4. *Other Carbohydrate Tests.* Sucrose has been used as a tolerance material in the study of absorption (see chapter on Malabsorption). Its uptake, however, may be dependent upon hydrolysis to glucose and fructose, and hence factors other than absorption are involved. Similarly, starch administration will raise the blood glucose level, but only after hydrolysis and absorption.

### Lipid Tests

1. *Oral Butterfat Tolerance Test.* A loading dose of butterfat (as heavy cream or butter) is given,[132] and blood lipid levels followed by a number of parameters (chylomicron counts,[133] increase in serum turbidity,[132] and chemical analysis for lipids). Even if all these procedures are carried out simultaneously,[43] the test is still limited by hepatic function, peripheral utilization, and the previously discussed factors affecting lipid uptake.

2. *Oral Vitamin A Tolerance Test.* Vitamin A is 'fat soluble' although the mechanism of its uptake may differ from that of other lipids. Water miscible vitamin A is seemingly handled in a manner similar to that of the 'fat soluble' molecule.[134] A large dose of the vitamin is given orally[132,135] and the four hour blood level compared with fasting values. In an alternate method, samples are drawn at several intervals after the oral dose and the resultant curve compared with a normal control. The curve is flat in sprue,[136] and does

not improve after therapy. Liver disease may also depress the blood levels of vitamin A after an oral dose.[137] Vitamin A may be briefly stored in mucosal cells before discharge into the circulation.[41] This may also tend to produce spurious results during the vitamin A oral tolerance test.

3. *Radioactive Oleic Acid Test.* Oleic acid, an unsaturated fatty acid, can be made to incorporate radioactive iodine ($I^{131}$). The resultant product can be detected by its gamma emissions[139] and is easier to use than oleic acid-$C^{14}$ (a weak beta emitter). The absorption of oleic acid-$I^{131}$ is independent of pancreatic function, and depends upon the competence of the mechanisms for extracting fatty acids from the lumen. The blood level of radioactivity may be followed after ingestion of a standard dose of the tagged fatty acid, or stool may be collected to determine the percentage excreted. This is but one of the tests presently available for evaluating intestinal function by means of tagged metabolites.[140]

### Amino Acid Tests

1. *Oral Glycine Tolerance Test.* The amino acid is administered in a dose of 1.0 to 1.7 grams per kilogram of body weight,[141] and blood samples withdrawn periodically. Blood levels are depressed in celiac disease as well as other conditions;[132] the test is apparently dependent upon normal hepatic function. Glycine absorption may not represent the uptake of all other amino acids, and caution has to be observed in interpreting the results.

2. *Oral Methionine Tolerance Test.* Following the ingestion of 1 gram per 15 kilograms of body weight,[142] the blood level is followed by a microbiologic technique. The method is difficult to standardize, and blood levels of methionine are affected by hepatic function as well as other factors.

3. *Other Tests.* Gelatin administration[141] or the ingestion of $I^{131}$-protein[143] are only indirectly tests of amino acid absorption, since they depend upon adequate digestion of the protein.

Absorption tests for iron, copper and vitamin $B_{12}$ are discussed in other chapters.

## CLEARANCE BY ALIMENTARY LUMEN CELLS*

Metabolites and fluids ingested via the oral cavity are transported or "cleared" from the gastrointestinal tract by cells lining its lumen.[151] An evaluation of alimentary function is thus essentially a description of the workings of the luminal cells. Though we possess little information as to the composition of these units, we can infer much as to their activity and efficiency by studying the "clearance" of materials from the intestine to the body proper.

The term clearance (C) has been defined,[152] in renal physiology, by the equation

$$C = \frac{UV}{P} \qquad (1)$$

where U and P are the urine and plasma concentrations of the compound, and V is the urine flow in ml/min. Thus, C is an expression of the number of milliliters of plasma that could be made free of the metabolite. Because there is no homogeneous fluid bathing each segment of the alimentary tract, this equation is not directly adaptable to an evaluation of gastrointestinal function. Moreover, a single measurement cannot adequately represent transport from the alimentary lumen. However, even with present crude technics, several parameters can be evaluated in studying the complex phenomenon of gastrointestinal clearance.

Static clearance ($C_s$) may be defined by the equation

$$C_s = \frac{Q_i - Q_e}{Q_i} \times 100 \qquad (2)$$

where $Q_i$ and $Q_e$ are the quantities ingested and excreted. The clearance figure thus obtained neglects the time factor (any interval may be designated); but, more fundamental, it is "static," for it gives little insight into the dynamics of the luminal cells. Rather, it represents the percentage of the ingested load taken up.

In the daily average body water economy, upon drinking 2,500 ml, one fifth of this quantity may eventually be passed by way of the fecal stream, and $C_s$=80 per cent. Hence, 80 per cent of the water was cleared by the gastrointestinal tract (although because of turnover and endogenously produced water, few of the excreted molecules may be identical with the ingested ones). In diarrhea, if the

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same quantity of water were taken, and 1,500 ml passed, $C_s$ would be 40 per cent.

Let comparative clearance ($C_c$) be determined by

$$C_c = \frac{\text{C pathologic}}{\text{C normal}} \times 100 \qquad (3)$$

Comparing the diarrheal and normal states (40 per cent/80 per cent x 100), $C_c$ is 50 per cent. Such a comparison would make is appear that in the diarrheal illness, luminal units were only 50 per cent as efficient as normal (possible mechanisms being rapid transit or cellular damage). However, even severe episodes of diarrhea do not reduce lumen cell function by as much as 50 per cent. This gross exaggeration tends to point out the difficulty encountered with a "static" figure, and brings us to a more dynamic evaluation of lining cell activity and efficiency.

Knownig the amount of product (P) brought to the alimentary lumen, and the material excreted (E) after a suitable time interval, the two terms are related by the expression

$$\mathbf{E = P + \Sigma\ (p + s - a)} \qquad (4)$$

where the summation figure includes the amounts presented to (p), absorbed by (a), and secreted by (s), the individual segments. Knowing P and E, we can calculate the total function of all segments for any substance we choose, and under any indicated conditions (such as before and after the tract is cleansed of bacteria); or, by studying the product at different levels of the tract, we can evaluate the contributions of the segments above our reference point. Generally, the summation figure will carry a negative sign, as greater quantities of a metabolite are absorbed, or subtracted, from the tract than are eventually excreted by it.

The major difficulty with the static clearance figure (and unfortunately, this is all we have available at the present for most nutrients) now becomes apparent. $C_s$ neglected the secretion of the luminal units in calculating the quantity of substance handled by the outermost portion of the lining cells. Equation 4 considered the function of the cell to be the uptake of materials appearing at the luminal surfaces, whether the compound came from the segments above or from its own secretions.

To illustrate: The 2,500 ml of water taken orally are but part of the fluid bathing the lining cells. For, in addition, the tract is also presented with an additional 8,200 ml by the secretory activities of the salivary glands, hepatic system, stomach, pancreas, and intestine.[153] Active clearance ($C_a$), which represents the action of the luminal units on the total material presented to them, may be defined, during any specified time interval, by

$$C_a = \frac{(Q_i + Q_p - Q_e)}{(Q_i + Q_p)} \text{ x } 100 \qquad (5)$$

where $Q_i$ and $Q_e$ are as before, and $Q_p$ represents the total quantity of the compound added to the lumen by the secretions of the lumen cells or the appendages of the alimentary tract. This expression can be presented in the terms of equation 4 as

$$C_a = \Sigma \frac{a}{p + s} \text{ x } 100 \qquad (6)$$

With a 2,500-ml water intake, an addition of 8,200 ml by the digestive organs, and a 500-ml fecal water loss, $C_a$ would be 95 per cent. Hence, 95 per cent of the water is reabsorbed, and efficiency approaching that of the renal nephron. For the diarrheal illness previously discussed, $C_a$ is 86 per cent. Comparatively, $C_c$ is 90 per cent, not 50 per cent as determined by the static clearance concept of equation 2, and we can infer that in terms of the total water presented to the lumen cells, some 90 per cent of normal is still absorbed in the pathologic state (assuming that the secretions of the appendages are unaffected by the diarrheal agent, or by substituting proper figures if they are altered). It will be observed that $C_c$ is a comparison between a pathologic and a normal state, and can be determined for any clearance parameter defined.

Additional information can be obtained as to lumen cell clearance by considering the alimentary tract and its appendages as a single unit membrane with two functions: (1) filtration of plasma to form gastrointestinal secretions; and (2) reabsorption of the filtrate plus ingested fluids and nutrients. In such a system the clearance gradient can be defined as

$$C_g = \Sigma \left(\frac{a}{s}\right) \qquad (7)$$

or

$$C_g = \frac{(Q_i + Q_p) - Q_e}{Q_p} \qquad (8)$$

For the normal state discussed, $C_g$ is 1.2 (that is, 1.2 times as much water is absorbed as is secreted), and in the diarrheal episode, 1.1. Total clearance ($C_t$), or the total amount of product handled by the lumen cells in both directions, is simply

$$C_t = \sum (s + a) \qquad (9)$$
$$C_t = (Q_i + 2Q_p) - Q_e \qquad (10)$$

If cardiac output were 2,500 ml of plasma per minute, and this flow was distributed with 30 per cent to the alimentary tract and 20 per cent to the kidneys, over a 24-hour period typical values of the clearance gradient and total clearance would be as calculated in Table 7. The percentage of flow filtered was determined from

$$\text{Percentage filtration} = \frac{\text{volume of secretion}}{\text{plasma flow}} \times 100 \qquad (11)$$

When one considers that the renal apparatus is designed for "losing" fluid from the milieu interieur, while the alimentary tract "gains," the clearance gradient values of 1.2 for the alimentary tract and 0.99 for the kidney, both so close to unity, loom all the more remarkable. They depict the narrow range within which homeostasis exists, and the function of lumen cells in the maintenance of this equilibrium.

TABLE 7

COMPARISON OF FILTRATION AND ABSORPTION FACTORS IN THE ALIMENTARY TRACT AND KIDNEYS

| ***Organ*** | *Plasma Flow ml/min* | *Per cent of Flow filtered* | *Cg* | *$C_+$ in ml per 24 Hours* |
|---|---|---|---|---|
| Almentary tract | 750 | 0.8 | 1.2 | $1.8 \times 10^4$ |
| Kidneys | 500 | 20.0 | 0.99 | $27.3 \times 10^4$ |

Such an analysis may be profitably employed in the study of disorders of alimentary tract transport (whether too great a clearance, as iron in hemochromatosis or copper in Wilson's disease, or too little clearance, as vitamin $B_{12}$ in pernicious anemia). It also may have applications in the evaluation of materials which are recycled through the gastrointestinal tract (as cholesterol and bile salts).

## COMMENTS ON USE OF THE WORD CLEARANCE

The term clearance has gained wide use in renal physiology to denote a volume of plasma rendered free of the substance under study. In this sense, clearance is an expression of volume and has not only a magnitude, but also an affixed volume designation (as ml). The use of the term in a different sense entirely, to indicate the efficiency of clearing substances from the gut lumen, may appear to be unwise. However, the word was chosen because it became necessary to avoid the overtaxed expressions absorption and uptake; these words have taken on the meaning of per cent intake, and this is precisely what we are trying to avoid. Absorption and uptake do not stimulate people to think; clearance used in this unusual sense may have some effect, and will be retained until another suitable word is suggested.

It is also possible to apply the clearance concept, in its original formulation for renal physiology, to a study of the intestinal tract. Thus, let L be the luminal concentration, P the plasma concentration, and A the alimentary·fluid volume per unit time. Then:

$$C = \frac{LA}{P} \qquad (12)$$

The difficulty arises in defining the quantities L and A. If we assume A is the fluid lost in the stool (500 ml/day), then for potassium:

$$C = \frac{15 \text{ mEq/L} \times 0.5 \text{ L}}{5 \text{ mEq/L}} \text{ or } 1.5 \text{ liters.}$$

Another interpretation is that L is the concentration of potassium actually in the stool (7 mEq/500 ml). Still an additional approach is that the 8,200 ml alimentary secretion is uniformly poured out over a 24 hour period, and that A is 8,200 ml/1440 minutes (5.7 ml per minute). Hence, if one were to apply the standard clearance formulation to the gut, great care would be necessary in defining the meaning of each symbol.

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# 10

# ELECTROLYTE SECRETION

WHILE MUCH attention has been paid to the intricacies of electrolyte absorption, relatively little work has been directed to unraveling the complexities of electrolyte secretion by the alimentary canal. However, a few generalizations are permissible.

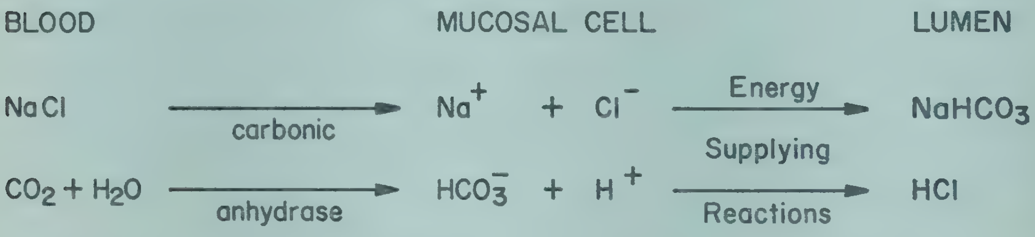
1. The process is often one of secretion, not excretion or diffusion. That is, passage of electrolytes into the lumen can occur despite a concentration gradient, and involves the expenditure of energy. For example, hydrochloric acid secretion by the stomach is inhibited by anoxia,[1] by reagents which combine with sulfhydryl groups,[2] and by inhibitors of carbonic anhydrase (an enzyme probably involved in the process [3]).

2. The controlling mechanisms are complex, and can adapt over a period of time to changing requirements. A dry diet calls forth secretions of one composition, while a diet with adequate water elicits a different response. Nervous, humoral and perhaps local factors are all operative. A new ileostomy may lose 300 milliequivalents of sodium per liter of fluid, whereas an established ileostomy loses but half as much sodium.[4]

3. The secretory ability is not uniformly distributed throughout the intestine. Bicarbonate secretion in the upper gut differs from that in lower regions;[8] indeed, two different types of secretion may be involved.[9] Discussions of some aspects of electrolyte secretion into the gut are given by Black[5] and Babkin.[6]

We picture fluids within the body as being subjected to rather narrow constraints on their pH, osmolarity, and volume. It is not so easy to think of such limitations to the composition of alimentary secretions, but they may indeed be operating (for, after all, alimentary fluids are directly derived from fluids of the milieu interieur).

*I. pH.* The pH of the jejunal secretion of man varies only within narrow limits.[9] This should not come as too much of a surprise, since digestive enzymes are maximally active within a specified pH region, and it is likely that buffer systems of the luminal contents (such as bicarbonate) maintain a well defined acidity or alkalinity. Bicarbonate may be the key factor in this pH regulation. The bicarbonate content of alimentary secretions can be much greater than that of the blood stream,[8,9] suggesting active secretion of $HCO_3$. Bicarbonate production, at least in the pancreas, is inhibited by compounds which interfere with the enzyme carbonic anhydrase.[10] Since inhibition of this enzyme also diminishes production of gastric hydroçhloric acid,[3] it likely plays an important part in the elaboration of alimentary secretions.



The role of the intestine in maintaining the pH of internal fluids is unknown. While absorption from limited segments of the gut may be unchanged by acidosis,[11] the effect of acidosis and alkalosis on intestinal secretions has yet to receive adequate study.

*II. Osmolarity.* Secretions of the digestive canal are isotonic or nearly so (see chapter on Isotonicity and the Dumping Syndrome). The statement has even been made that feces are isotonic.[7] Since the amount of sodium excreted in stool is rather slight (10 to 125 mg) despite variations in oral intake,[12,13] there may well be systems in the lower intestinal segment which are active in secreting or absorbing other electrolytes,[19] to retain a semblance of isotonicity. Whether there is an obligatory stool loss of certain electrolytes (as potassium), such as occurs in the urine, is uncertain but appears possible.[14] Dietary intake of electrolytes also affects the quantity of electrolytes in the ileal contents;[14] however this may have little significance in net electrolyte loss from the body because of colonic reabsorption.

*III. Volume.* The volume of alimentary secretion is variable; it varies from person to person, varies with dietary intake, and varies with the quantity of water ingested. Since there is a certain commitment to approximate isotonicity within the gut lumen (although not an absolute commitment), the volume of water is fairly set once the quantity of electrolyte is specified. Water within the gut lumen (electrolytes as well) is in rapid interchange with its counterpart in the blood stream (and intraluminal electrolytes are included in determinations of 'exchangeable electrolytes' within the body). Approximate values for the volume of digestive secretions per day, as given by Gamble,[15] are shown in Table 8.

TABLE 8

DAILY VOLUME OF ALIMENTARY SECRETIONS

| *Secretion* | | *Volume (ml)* |
|---|---|---|
| Salivary | | 1,500 |
| Gastric | | 2,500 |
| Biliary | | 500 |
| Pancreatic | | 700 |
| Intestinal | | 3,000 |
| | Total: | 8,200 ml |

Adapted, by permission, from Gamble, J. L.: Chemical anatomy, physiology and pathology of extracellular fluid. Cambridge, Mass., Harvard University Press, 1950. 5th edition. Chart 37.

The magnitude of the total secretion (8,200 ml) can be appreciated when it is noted to be over twice as large as the average blood plasma volume (3,500 ml). When we also consider that 2,000 ml of water may be taken in daily in liquid form, or as part of food, the large volume (10,200 ml) of fluid which must be reabsorbed is apparent. All but 500 ml of water (or less) are absorbed and prevented from leaving in the stool. This is an absorptive efficiency of (10,200-500)/10,200 or 0.95. It is obvious that an efficiency much less than this would result in excessive loss of fluid from the body.

As we have mentioned, the electrolyte composition of alimentary fluids is somewhat variable. The values in Table 9, however, are indicative of the approximate magnitudes to be found in the healthy adult.

TABLE 9

AVERAGE ELECTROLYTE COMPOSITION OF ALIMENTARY SECRETIONS

| *Source* | *mEq/L** | *Na* | *Cl* | $HCO_3$ | *H* | *K* |
|---|---|---|---|---|---|---|
| Saliva | 120 | 100 | 80 | 20 | — | 20 |
| Gastric juice | 148 | 19 | 131 | — | 120 | 9 |
| Pancreatic secretion | 157 | 145 | 38 | 115 | — | 5 |
| Bile | 152 | 142 | 107 | 32 | — | 5 |
| Jejunal secretion | 151 | 142 | 115 | 31 | — | 9 |
| Blood | 155 | 142 | 103 | 27 | — | 5 |
| Daily dietary intake | — | 99 | 114 | — | — | 77 |

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*Milliequivalents per liter (anion or cation).

Small amounts of electrolytes are found in the stool (about 7 mEq of potassium, and 3 mEq of sodium). In diarrheal states, sodium loss increases and may be severe enough to result in a decrease in the blood sodium level. Other electrolytes may also be lost in excessive amounts in diarrheal stools.

During the passage of digestive fluids and ingested materials down the alimentary tract, an orderly series of events occurs so that the final fecal mass does not carry excessive water, electrolytes or nutrients from the body. Interference with these sequential steps can result in drastic loss of materials. During intestinal obstruction for example, the disordered water and electrolyte balance can be as serious a problem as the underlying disease itself. In the broadest sense, intestinal obstruction exists when any event prevents the ordered progress of materials from mouth to anus, and the regulated exchange of fluid, electrolytes and nutrients across the mucosa. Thus, an individual with malabsorption may not have a gross anatomic block across the gut, but still loses excess materials in the stool (this is generally considered a mucosal obstruction, rather than frank intestinal obstruction).

The causes of intestinal obstruction are legion. By far and away the commonest responsible factors are mechanical (such as occlusion of the lumen by strangulation of a hernia, volvulus and intussusception). Recognition of intestinal obstruction is of great importance early in the development of the condition; survival is inversely related to the delay in diagnosis. When obstruction occurs, fluids accumulate above the affected point, producing distention and embarrassment of the gut wall and its blood supply. In addition, depending upon the level of obstruction, fluids may be lost through vomiting. Therapy is directed at removing the cause of obstruction, restoring continuity of the alimentary canal, and replacing fluids and electrolytes lost through vomiting and other causes. We will not further discuss this important topic, as three well known monographs exhaustively deal with the subject.[16,17,18]

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# 11

# INTESTINAL BACTERIA AND THEIR PRODUCTS

FROM THE TIME the newborn infant swallows air, bacteria become permanent residents of the gastrointestinal canal. The presence and activity of these organisms has received much attention and speculation, ranging from the view point that their products might intoxicate the unhappy host, to a realization that enteric bacteria serve multiple functions. It is now recognized that intestinal bacteria synthesize a number of vitamins (in fact bacteria or related forms are the ultimate source of all the natural vitamin $B_{12}$ in the world) . In addition, they aid in the digestion of otherwise unassailable food materials, not only in ruminants, but in other animals as well.[1]

Except in unusual instances of septicemia or ascending infection of the umbilical cord, bacteria enter the digestive canal of the newborn via the mouth and anus. The flora of the infant's gut consists principally of gram-positive microbes, such as Lactobacillus acidophilus. These acid-producing species perhaps account for the sour odor of the infant's stool. The Lactobacillus population is favored more by a diet of human milk than one of cow's milk[2] (as human milk contains an oligosaccharide which promotes the growth of these bacteria[62]) ; this may have little significance in vivo, and attempts to favor or hinder the growth of intestinal bacteria in the newborn[3] appear to have little validity. On occasion, one organism predominates in the infant's alimentary canal, but therapy is usually withheld unless there are symptoms or the organism is considered best eradicated for other specific reasons.[4]

At the time the infant's diet is enlarged to include solid foods, gram-negative bacteria begin to appear in large numbers and become well established intestinal species. Adult stool bacteria probably represent the type of organisms residing in the lower colon, and we may conclude that various coliforms (Gram-negative) and enteric cocci (Gram-positive) are the most common inhabitants. Stool bacteria counts reveal between $1 \times 10^{11}$ and $4 \times 10^{12}$ organisms per gram of fecal dry weight, with the coliforms predominating.[5] It is usually difficult, and clinically unprofitable, to attempt to identify all organisms present in stool specimens. When pathogenic forms are suspected, differential cultures and fermentive reactions serve to identify the bacteria. Care must be taken, however, in diagnosing intestinal tuberculosis. While it is true that the tubercle bacillus may invade the gut wall[6] (perhaps by hemic spread), more often the presence of these organisms in the stool simply means that they have gained access through swallowed saliva. In children, since almost all saliva is swallowed, stool examination and culture may reveal the mycobacterium.

The average daily fecal output is 100 grams, although there are wide variations. Two-thirds or more of fecal material is water; of the solid substances, bacteria constitute 25 per cent. Thus, an adult excretes approximately 8 grams of bacteria daily (100 x 1/3 x 1/4). Instances of constipation are often accompanied by a reduction in the fecal water content and an increase in the bacterial population; conversely, increased water intake may decrease the fecal output of bacteria.[57] The largest quantity of stool microbes are nonviable, perhaps killed by antibiotic and metabolic products of their neighbors. However, the surviving organisms are still active, and while the outside of a formed stool registers pH 7, the center can be at pH 5 because of organic acids generated. Fecal production continues when there is no oral intake. It can even be observed in isolated intestinal loops. The fecal-like material produced by an isolated gut segment is composed of substances from the blood, sloughed cells and bacteria. The components of the intraluminal mass resemble a protein-free blood filtrate that has been altered by bacterial action (such as coprosterol production from cholesterol).

It should also be recognized that many microscopic and submicroscopic species attack the gut, in addition to the usual pathogenic bacteria. Strains of E. coli may produce intestinal symptoms,[7,8] as may viruses,[9,10] trypanosomes,[11] and Histoplasma capsulata.[12] Indeed, the logical question to ask is why the intestine is normally resistant to these organisms. The small intestine apparently produces a substance which inhibits bacterial growth;[13] loss of the substance precedes the appearance of bacteria in the upper small intestine. A large factor in preventing bacterial entry is undoubtedly found in the mucosa itself. Following sigmoidoscopy for instance, rarely can bacteria be cultured from the blood stream despite trauma to the lower bowel.[14] Damaging the mucosa enhances the pathogenicity of bacteria within the gut lumen,[15] and it is known that certain bacterial toxins interfere with the metabolism of luminal cells[16] (perhaps rendering them more susceptible to invasion). In some instances the absorption of tagged bacteria from the lumen has been demonstrated.[17]

Bacteria are found in abundance in the large bowel, but their number diminishes as the more cephalic portions of the alimentary tract are approached. There has been interest in the number and type of bacteria in the small intestine because of the demonstration that some cases of malabsorption are improved following use of antibiotics which reduce the intraluminal bacterial population (see chapter on Malabsorption). However, no distinct difference has yet been noted between intestinal bacteria of normal individuals and those with malabsorption.[18] The healthy stomach is practically bacteria free in man, but not in all animal species.[19] Part of the gastric protection may be found in the great acidity of its secretion, as well as the presence of lysozyme in salivary fluid (which destroys some bacteria). In cases of fistula, severe dehydration or obstruction, bacteria invade the gastric lumen. Great emphasis was formerly placed on the finding of gram-positive Boas-Oppler lactobacilli, and lactic acid, within the gastric contents in cases of gastric carcinoma. This, however, is an uncertain diagnostic aid and generally occurs too late to be of assistance.

## BACTERIAL PRODUCTION OF NUTRIENTS

Because of the large number of enzyme systems present in the intraluminal bacteria population, it is not surprising that some of the end products are suitable for human nutrition (while others are unusable or even noxious).

When maintained on a diet devoid of B complex vitamins, rats usually lose weight and die. Fridericia[20] observed that when large numbers of rats were so treated, a few of the animals survived and gained weight. The survivors had pale and bulky stools, and this was traced to the intestinal flora. The phenomenon, termed *refection,* was supposedly due to the ability of intestinal organisms to synthesize the vitamins B, especially while the animals were on a high starch diet. These experiments were performed over thirty years ago, and we can question whether the diets were truly deficient in all components of the B complex. Additional evidence, however, indicates that starch and dextrins support the growth of bacteria capable of synthesizing a number of B vitamins, while sucrose does not maintain this activity nearly as well. Such observations are open to host strain differences as well as qualitative and quantitative variations in the intestinal flora.[21] More definitive work is needed as to the amounts produced and their availability for absorption. As long as coprophagy is allowed in the rat, many of the B vitamins can be omitted from the diet. Apparently stool bacteria manufacture the vitamins.

Present knowledge of the synthesis of vitamins by intestinal bacteria has been summarized by several authors;[22,23,24] Williams et al[25] provide an interesting table showing the ability of numerous bacterial species to produce different vitamins. In man, the output of certain B vitamins may be greater than the dietary intake, suggesting synthesis of additional amounts by intestinal bacteria and absorption of the materials.[26] Studies on germ-free animals may be helpful in delineating the extent of host dependence on the synthesizing abilities of bacteria. For instance, the synthesis of methyl groups (necessary for the production of creatine, epinephrine, thymine and other compounds) is not dependent upon organisms of the gastrointestinal tract.[27] The function of alimentary bacteria in degrading vitamins is not clear at present (see discussion of thiaminase in chapter on Vitamins). Certain bacteria of the digestive tract require the presence of vitamins for growth,[40] and perhaps reduce the amount of available vitamins. Recently an extensive summary of germ-free vertebrates has appeared; this contains many references to the intestinal tract and its alterations in the germ-free state.[63]

## BACTERIAL PRODUCTS IN FECES

Aside from constituting a large part of the bulk of feces, bacteria also contribute to its color (by producing stercobilin from bilirubin), its lipid content (coprosterol, made from cholesterol), and its odor (decarboxylation products of amino acids). The total number of reactions carried out by intestinal bacteria are too numerous to consider in detail, but the major ones will be listed.

### Stercobilin

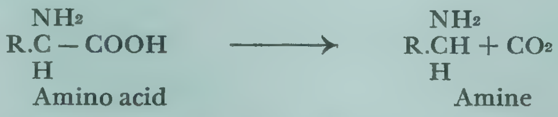
Hemoglobin is degraded in the body to verdohemoglobin, then: ⟶ biliverdin ⟶ bilirubin. In the intestine, probably through the action of bacteria, the following reaction occurs: bilirubin ⟶ mesobilirubinogen (and destruction of intestinal bacteria by antibiotics inhibits bilirubin degradation[60]). This, in turn, is changed to stercobilinogen and stercobilin. Stercobilinogen is identical with urobilinogen (which, in combination with a peptide, produces the characteristic color of urine). Some stercobilinogen escapes conversion to stercobilin, is absorbed from the gut, picked up by the liver and reexcreted via the bile. The small amount of stercobilinogen that is not sequestered in the liver is excreted by the kidneys (hence the origin of urobilinogen). More recent views on the urobilin materials and their distribution are given by Watson,[64] and others.[65]

### Lipid Alterations

Intestinal bacteria can convert fatty acids to the corresponding alcohols (such as cetyl alcohol from palmitic acid); there may be a mechanism in the mucosa for reversing this change.[28] Intraluminal organisms can also alter the degree of saturation of fatty acids by hydrogenation reactions.[29] Cholesterol entering the gut from the diet, bile, or secretion across the mucosa, is converted to coprosterol (by way of cholestenone and coprostenone) in large amounts by bacteria. The effect of this is to reduce the amount of sterol available for absorption; whereas cholesterol is able to pass the mucosa in both directions, coprosterol can not be taken up, and passes out in the feces. Another reduction product of cholesterol, cholestanol, also appears in the stool; its presence, however, is due to hydrogenation reactions within the body and not primarily from intestinal microbes.

**Amino Acid Decarboxylation**

In animal tissues, the metabolism of amino acids generally involves deamination before decarboxylation. In bacteria, this order may be reversed.



$$\underset{\text{Amino acid}}{\overset{NH_2}{\underset{H}{R.C-COOH}}} \longrightarrow \underset{\text{Amine}}{\overset{NH_2}{\underset{H}{R.CH}}} + CO_2$$

The resulting amines are common in the fecal mass. The diamines, cadaverine and putrescine, were noted many years ago[61] (they are formed from lysine and ornithine) . Formation of enzymes catalyzing these reactions appears to be favored in an acid media.[30] In addition, the enzymes are adaptive, being produced only when a suitable substrate is present in the medium. The number of decarboxylating enzymes produced by intestinal bacteria is large; a partial listing is given in Table 10, adapted from data presented by Fruton and Simmonds.[31]

TABLE 10

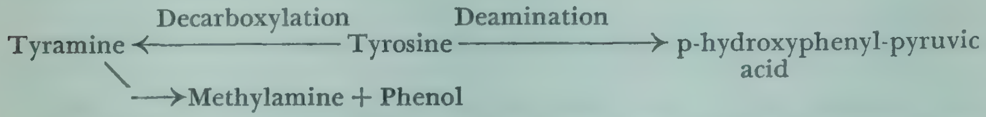
REPRESENTATIVE BACTERIAL DECARBOXYLASES

| *Microorganism Producing the Enzyme* | *Amino Acid Acted Upon* | *Resulting Amine* |
|---|---|---|
| B. cadaveris | L-lysine | Cadaverine[32] |
| E. coli | L-arginine | Agmatine |
| Cl. welchii | L-histidine | Histamine |
| | L-glutamic acid | $\gamma$-aminobutyric acid |
| Cl. septicum | L-ornithine | Putrescine |
| S. fecalis | L-tyrosine | Tyramine |
| | L-phenylalanine | Phenylethylamine |

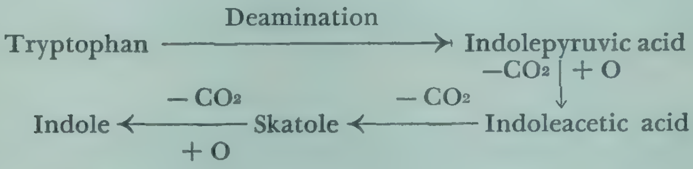
Adapted, by permission, from Fruton, J. S.; Simmonds, S.: General Biochemistry. New York, John Wiley & Sons, 1953, p. 672.

**Deamination and other Amino Acid Reactions**

Some species of intestinal microbes deaminate amino acids either as an independent step or coupled with other alterations of the substrate. Typical deaminations convert tryptophan to indolepyruvic acid, and tyrosine to p-hydroxyphenylpyruvic acid. Such reactions liberate ammonia which can be absorbed across the mucosa (see chapter on Enterohepatic Circulation). The products of these reactions, and of decarboxylations, may be further degraded. For example, tyrosine undergoes the following events when attacked by gut bacteria.



An interesting series of reactions are produced by bacteria working on the amino acid tryptophan. The end product, indole, is absorbed from the gut and converted to indoxyl in the liver. Upon conjugation with sulfate; the potassium salt is formed (indoxyl an ethereal sulfate) and passed in the urine. This is a liver function; certain sulfatases however occur in the gut[58] and may be due to mucosal cells as well as bacteria of the lumen.[59] Skatole behaves in identical fashion, forming skatoxyl and the corresponding ethereal sulfate. Indole and skatole, as well as their esters, are not products of intermediary metabolism in man, but rather represent substances produced entirely by bacterial action. Tests for indole, skatole and indoxyl [33] enjoyed use a number of years ago when it was believed they offered an index of "intestinal putrefaction." There appears, however, to be little correlation between the formation of these substances and intestinal function. More recently Dalgliesh and coworkers [34,35] have shown that indole and its derivatives in the gut may be further acted upon by the body with the formation of products such as N-hydroxyskatole-o-sulfate. The significance of such observations is that some of these compounds are toxic to cells, their genesis depends upon production of their precursors by intestinal bacteria, and their excretion can be eliminated by antibiotics which inhibit the growth of alimentary microorganisms. Whether such products have importance in the production of the malabsorption syndrome is unknown (urinary excretion of these materials in patients with malabsorption was eliminated after the use of antibiotics).



### Other Reactions

Various bacteria within the gut lumen can degrade bile acids,[54] convert choline to trimethylamine,[36] produce hydrogen sulfide from sulfur compounds,[37] reduce organic nitro compounds,[38] make benzoic acid from phenylalanine, and attack food materials with the generation of methane and other hydrocarbons. Bacteria may also be of importance in the production of substances which pigment the bowel wall during use of cascara derivatives (see chapter on Pigmentary Disorders) and may account for alteration of steroids in the diet.[41]

The production of noxious agents within the alimentary lumen is not synonomous with body injury. This is because only small amounts are usually evolved, and the intestinal wall protects against their passage. For instance, introduction of large amounts of histamine into the intestine does not produce symptoms as long as the mucosa is intact (perhaps due to the ability of the intestinal enzyme histaminase to break down histamine). However, damaging the mucosa by detergents is followed by the absorption of intraluminal histamine and the production of toxicity.[39] Many of the phenomenon formerly attributed to 'intestinal autointoxication' (headache, malaise) may be duplicated by distention of the rectum and probably are pressure manifestations rather than a reflection of toxic product absorption.

## ANTIBIOTICS AND INTESTINAL BACTERIA

Antibiotics can be divided into two large classes—those which are absorbed from the gastrointestinal tract, and those which are not. The latter group may be effective in reducing the intraluminal microbe population but has little systemic action. Preoperative cleansing of the intestine has been studied by Poth;[42,43] he has established the requirements an acceptable antiseptic for preoperative intestinal cleansing must fulfill, and has recommended the use of neomycin and sulfathalidine. Experimentally, postoperative intraluminal injection of antibiotics aided the recovery of dogs subjected to colon anastomosis,[44] presumably by protecting partially devascularized areas and preventing leakage of bacteria.

Following oral administration of nonabsorbed antibiotics, the intestinal microorganism population may be altered so that:

A. Fungi such as Candida albicans overgrow, or

B. Gram-positive forms (as staphylococci) assume preponderance.

When such events occur, discontinuance of the antibiotic is necessary and attempts are made to restore the normal flora. Some authors have advocated the use of lactobacilli along with antibiotics,[45] so that the flora will not be too seriously deranged. The use of enemas composed of stool homogenates from normal persons have also been advocated to restore a bacterial flora altered by antibiotics.[46] On occasion, stool smears will show gram-positive forms (such as staphylococci) to be in the majority. Clinical symptoms of intestinal irritation and mucosal damage often follow (this is discussed elsewhere under the heading of Pseudomembranous Enterocolitis).

Antibiotics have other as yet little understood effects on the intestine. For instance, mice treated with oral streptomycin are more susceptible to infection by Salmonella enteriditis,[47] perhaps because of an altered gut flora. Alimentary bacteria also play a role in the development of certain forms of shock (see chapter on Blood Supply and Gastrointestinal Hemorrhage); their effect can be lessened by the use of antibiotics. Intestinal bacteria provide a challenge to hepatic mechanisms (either by the products they produce or by actual entry into the portal circuit), and liver damage may be partially prevented by the use of nonabsorbed antibiotics.[48] Antibiotics also have a stimulant effect on the growth of animals,[49] and reduce the weight of the chick's intestinal tract.[50] The mechanism of antibiotic stimulation of growth is unknown, but three factors have been discussed.

1. Destruction of microorganisms which are detrimental to the host;
2. Changes in the flora which favor the propagation of bacteria which synthesize growth promoting factors;
3. Inhibition of the growth of bacteria which compete with the host for essential nutrients.

Goldsmith[24] states that there is evidence for the first two of the above possibilities and summarizes the data. While under certain circumstances antibiotics spare various vitamins in man, under different conditions, they lead to vitamin deficiency (perhaps by the destruction of bacteria which also synthesize the vitamin, by intracellular competition of host enzyme systems, by diminished intake through anorexia and vomiting, and by the overgrowth of new organisms and the occurrence of diarrhea). That antibiotics generate vitamin deficiencies is a real possibility.

Studies of the metabolism of the intestine are complicated by the fact that observed effects may be due to contaminating microorganisms.[19] Great care must be observed, however, when antibiotics are administered to obviate these effects. While in some cases antibiotics do not interfere with intestinal processes,[51] in other cases they do.[52,55,56] No general rule can be given at present.

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## 12

# BLOOD SUPPLY AND GASTROINTESTINAL HEMORRHAGE

ARTERIAL BLOOD is brought to the gastrointestinal canal by means of the celiac, superior mesenteric, and inferior mesenteric arteries (Figure 3). Venous drainage is into the portal system and is discussed in the following chapter. To reach the gut, vessels traverse the mesentery, and extensively branch to supply all areas of the viscus. The degree and type of branching (vasa rectae) are fairly

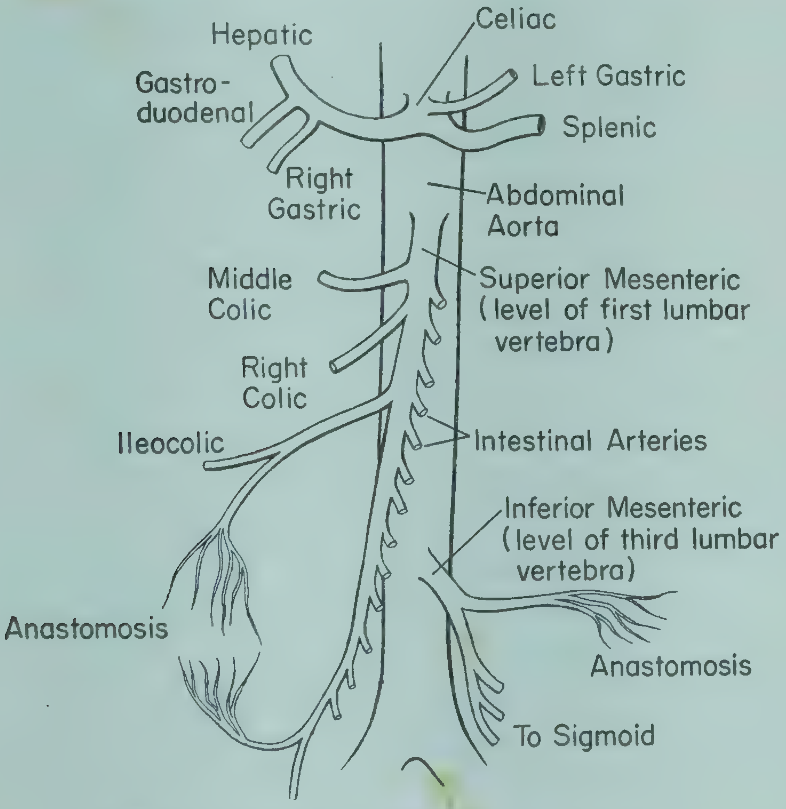
Hepatic
Celiac
Gastro-duodenal
Left Gastric
Splenic
Right Gastric
Abdominal Aorta
Middle Colic
Superior Mesenteric (level of first lumbar vertebra)
Right Colic
Ileocolic
Intestinal Arteries
Inferior Mesenteric (level of third lumbar vertebra)
Anastomosis
Anastomosis
To Sigmoid

Figure 3. Arterial blood supply of the intestine.

characteristic for each section of intestine and thus may be employed, during operations, to approximately identify the area of gut in the surgical field. Variations and anomalies of vessels to the intestine are not unusual. Of particular interest is the intestinal obstruction resulting from an aberrant superior mesenteric artery crossing the duodenum.[1] The arrangement and collateral patterns of the mesenteric vessels have been extensively studied,[2] since surgical removal of part of the vascular bed may be occasioned by neoplastic disease or other maladies.

The competence and functional reserve of mesenteric arteries has aroused interest because of cases of apparent insufficiency. Dunphy[3] reviewed the records of 12 individuals who died of occlusion of the mesenteric vessels, and noted that seven of them had recurrent abdominal pain for weeks, months, or even longer. Derrick and Logan,[4] reported 28 out of 75 necropsies showed narrowing of the superior mesenteric artery, caused by arteriosclerosis. Symptoms apparently arise when the vessels become sufficiently compromised and a vigorous blood flow is demanded (such as postprandially). The condition called "intestinal angina" is often characterized by cramping abdominal pains, 30 to 60 minutes after meals.[5] The genesis is viewed as intermittent ischemia, due to narrowing (or perhaps only spasm), of vessels to the intestine. Relief is sometimes obtained by small feedings or by lying down after meals (questionably by lessening traction on the vessels or by a change in hemodynamics). Diagnosis is difficult as the pain may mimic that of peptic ulcer. Therapy is nonexistent except for surgical anastomosis if a large vessel is occluded.[6] Arteriography has a questionable diagnostic role to play here as well as following surgery upon the mesenteric vessels (to insure their competence [7]). However, major complications can follow the introduction of radiopaque material into abdominal vessels.[8,74] The syndrome of intestinal angina resembles closely the pain pattern seen in certain individuals with essential hyperlipemia. Perhaps in the latter condition, after fat ingestion, the lipid content of the blood stream increases markedly and in some way interferes with the ability of abdominal vessels to transport sufficient oxygen.

Vessels to the intestine can be involved in aneurysm formation, and rupture of the aneurysm into the intestinal tract may rarely occur.[9] Vascular paths to the gut are also embarrassed by the lesions of Buerger's disease (thromboangiitis obliterans [10,11]). Blood channels within the gut itself may be afflicted with varices,[12] hemangiomatosis,[13] and telangiectasia.[83] Blood may leak not only into the lumen, but also into the intestinal wall itself.[14,15] Various purpuras also affect the intestine; a syndrome first outlined by Edouard H. Henoch in 1868 consists of purpura, gastrointestinal symptoms (such as colic, blood diarrhea), and joint pains. Also rarely, vessels of the gut are affected by lesions of Kaposi's hemorrhagic sarcoma, which usually has more peripheral manifestations.[75] It should be clearly recognized that these entities produce bleeding by means of errors of the vessels or the coagulation mechanism. The most common cause of gastrointestinal hemorrhage, however, is due to erosion of a blood vessel by a peptic ulcer.

Severe occlusion of vessels to the intestine leads to infarction. Both emboli[16,17,18] and thrombi[19] are capable of blocking mesenteric blood channels, with catastrophic results. Surgical intervention can be successful if the condition is recognized soon after its occurrence.[16] In some cases of bowel infarction, an obstructing lesion in the vessels can not be demonstrated; it has been postulated that severe cardiac failure perhaps aided by vessel spasm produces ischemia severe enough to result in tissue destruction.[20] "Sludging" of blood has been shown to occur in the viscera,[23] and this too compromises the circulation. Venous thrombosis may also produce infarction of the bowel,[21] as may gastrointestinal invasion by the fungus Rhizopus (the agent responsible for the clinical syndrome called Mucormycosis [84]). On occasion, signs and symptoms of intestinal infarction are atypical and the condition mimics other disorders.[22] Diagnostic procedures, including studies of peritoneal fluid during bowel infarction are discussed by Gray and Amador.[21] Levine and Warren [85] produced intestinal infarcts, by the injection of antibodies. The role of vasoconstrictors in gut damage has been mentioned.[91]

Relatively few biochemical parameters have been evaluated during or immediately following intestinal infarction. During the acute phase of intestinal damage, enzymes formerly located intracellularly gain access to the blood stream. There is a rise in serum transaminase values during experimental intestinal infarction in dogs.[24] In man during small bowel necrosis, amylase values often increase in the serum, indicating either pancreatic damage or the liberation of an amylase from the intestine proper [25] (an amylase can be histochemically demonstrated in the intestine [26]). Of perhaps great importance in future studies of malabsorptive diseases, is the demonstration that following bouts of intestinal ischemia in man, malabsorption may result.[19]

## STUDIES OF MESENTERIC AND INTESTINAL VESSELS

Passing as they do through the semitransparent mesentery, vessels to the intestine can be studied microscopically in living animals by the use of suitable techniques.[23,27] During intestinal contractions, vessels in the gut wall are passively contracted.[28] Vessels to, and probably in, the intestine react to histamine,[29] and other hormones, as shown by microscopic and other studies. Blood channels in the mesentery can also be investigated by the introduction of micropipettes; using this technique, Landis[31] found pressure values ranging from 10 to 40 cm of water, depending upon the species studied.

Vessels to the intestine must maintain a flow of oxygen to the rapidly metabolizing mucosal cells. When the oxygen content of blood falls below 12.8 ml per 100 ml (normal value: 20), both tonus and motility of the gut are adversely affected.[32] Blood passing through the intestine can be altered in composition as absorbed nutrients enter and certain substances are used by the mucosal cells themselves. In addition, on its way through the intestine, blood may pick up substances affecting the degree of constriction of blood vessels,[30,45] and may have some of its amino acids transaminated.[33] Various other parameters have been studied, such as total splanchnic blood flow and heat production,[34,35] but have not yet been sufficiently correlated with clinical alterations.

## QUANTITATION OF INTESTINAL BLOOD FLOW AND PRESSURE

Only in recent years have reliable studies on the quantitation of blood flowing to, and pooled in, the intestine been reported.

These results are of great interest and challenge long held beliefs that the splanchnic area is a potential 'pool' for blood. For instance, Reininger and Sapirstein[36] reported that increased blood flow to the gut, after feeding, did not draw blood away from other areas, but was simply the result of an increased cardiac output. The sequestration of blood in the splanchic area is not a critical factor in the development of irreversible shock,[37,38] and the actual weight of the gut and its blood may decrease during hemorrhagic hypotension.[39,78] Indeed, experimentally it has been noted that perfusing mesenteric vessels with blood, after dogs have been bled, prevented the development of irreversible shock (suggesting that the gut too is deprived of an adequate blood supply rather than being the site of blood pooling). Intestinal bacteria or their products undoubtedly contribute to the mortality following hemorrhage.[79] Data have been presented that hypothermia may be useful during bouts of mesenteric vascular occlusion.[86] The intestine has also been shown to play a small role in the disposal of leukocytes; [87] how this is affected in disease states is unknown.

A large quantity of blood is delivered to the abdominal viscera with each beat of the heart; the largest portion of this flow is directed into the renal and hepatic circulations. The actual amount of blood entering the large and small intestine is influenced by such factors as changes in posture, and is probably not a static figure (in dogs, an average blood flow is 0.31 ml/gram intestinal tissue/minute [80]). The volume of blood within the splanchnic vessels and the intestinal wall itself also depends upon multiple factors, including carbon dioxide pressure in blood. Overventilation, leading to a lowering of $CO_2$, is accompanied by a fall in blood pressure,[40,41] perhaps due in part to vasodilatation in the splanchnic region. There is also evidence that blood within the intestine has a lower hematocrit than general arterial blood.[81] Studies on the chicken have shown that the gut receives 1.1 ml blood/gram tissue/minute, or 17.4 per cent of the cardiac output.

During exercise, it has been assumed that blood is drained away from the splanchnic area; this concept has been challenged[42] and additional work is needed.[76] Rapid changes in the caliber of splanchnic vessels are probably a function of the sympathetic nervous system; upon vascular distention, afferent impulses are initiated from pacinian corpuscles in the mesentery.[43] Splanchnic nerves to the viscera[44] also play a role in maintaining blood pressure (as witnessed by the fall in pressure following splanchnicectomy). The relationship between the amount of intestinal blood flow and the ability to absorb nutrients has been little studied, although some work is available on the hemodynamics of intestinal circulation in terms of pressure and flow [46] (in dogs, a minimum pressure of 16 mm Hg was necessary to have blood flow through the intestine).

Resistance is defined by the relationship:

$$\text{Resistance} = \frac{\text{pressure gradient}}{\text{flow}}$$

If the centimeter-gram-second system of measurement is used, the unit of resistance is $(\text{dynes}/\text{cm}^2) / (\text{cm}^3/\text{sec})$, or dynes second $\text{cm.}^{-5}$ When the pressure gradient is measured in mm Hg, the conversion factor 1332 is necessary. By analogy to other systems, we may define small intestinal resistance:

$$\text{Small intestinal resistance} = \frac{\text{Mean superior mesenteric artery pressure x 1332}}{\text{Flow into the vessel}}$$

Studies of the magnitude of intestinal resistance have not been reported, although it is known that the general splanchnic vascular resistance is elevated in patients with coarctation of the aorta.[48] Multiple capillary beds of the intestine undoubtedly add to the resistance to flow. Data on vascular resistance can also be expressed in 'Wood units,'[47] defined as:

$$\text{Resistance} = \frac{\text{Mean vessel pressure (mm Hg)}}{\text{Flow in liters/minute}} .$$

This simplified expression for resistance has been applied to studies of the lung, and should be equally applicable to the gut.

## BLOOD WITHIN THE ALIMENTARY LUMEN

The presence of blood within the alimentary lumen indicates either bleeding into the area or the swallowing of sanguinous fluid (such as occurs after bleeding into the oropharynx). Coagulation defects, vascular abnormalities (as esophageal varices), and injury to tissue may all result in the entrance of blood into the lumen.

Clinically, the commonest causes of gastrointestinal bleeding are peptic ulcer, gastritis, gastric carcinoma, and tumors of the large bowel. So common are the latter two lesions that the most frequent cause of iron deficiency anemia in the aged can be traced to chronic gastrointestinal blood loss.[49,50]

Gastrointestinal bleeding is best classified for clinical purposes by the site of blood leakage and the quantity lost. Determination of both site and quantity is sometimes exceedingly difficult, and poses major problems in the management of alimentary bleeding. A useful clue may be the orifice from which blood is lost, and its color. Four descriptive terms are employed:

Hematemesis: vomiting of bright red blood;
Hematachezia: passage of bright red blood in the stool;
Melenemesis: vomiting of darkened blood;
Melenachezia (or melena) : passage of darkened blood in the stool.

Passage of blood in the stool is far more frequent than the vomiting of blood. Hematemesis, however, is a fair indication that the lesion is high in the alimentary canal. Hematachezia is generally due to bleeding low in the intestine; this is not an infallible rule, since entrance of blood into the gut may act as a trigger for rapid emptying.[51] It is possible that color of blood in the stool depends more on the time the sanguinous material has been in the lumen than on the level of bleeding.[52] Tarry stools occur after as little as 50 ml of blood have passed into the lumen;[53] after a major blood loss (over 1,000 ml), black material may be passed for several days even if additional hemorrhage is not occurring.[51]

Several procedures may aid in establishing the site of gastrointestinal bleeding. Gastroscopy and proctoscopy are useful in ruling out bleeding at the extreme ends of the gut. The loss of blood from esophageal varices is often the result of hepatic disease, which can be associated with diagnostic elevations of the blood ammonia level.[54] When bleeding occurs between the pylorus and rectosigmoid, there is no entirely satisfactory method of evaluation. Roentgenographic examination of the digestive canal without trauma to the abdominal wall (Hampton technique), during the phase of acute bleeding, may reveal the site of bleeding.[55] Another procedure used quite often is the string test (Einhorn). A string is swallowed, and withdrawn after being in the digestive canal for a period of time. The point on the string at which blood appears is taken as the length down the canal at which bleeding is taking place. The method suffers from two disadvantages.

1. Blood in the intestinal lumen does not stay at the point of origin, but widely distributes itself up and down the gut. The appearance of blood at a certain level of the string does not necessarily mean that bleeding occurred at that point; it rather signifies that the uppermost region of excursion of the blood, at the time of the test, has been found.

2. Strings taken by mouth have a tendency to curl up, especially in the stomach. Even if blood is found on the string, it may not be possible to correlate this with the exact level of bleeding. Because of these limitations, Rappaport[56] suggested that the string be marked with a radioopaque material so that its passage could be followed by x-ray. More recently, Traphagen and Karlan[57] proposed a fluorescein string test for the localization of upper gastrointestinal hemorrhage. Fluorescein is given intravenously after a string is passed orally; the string is then withdrawn and examined for both blood and the fluorescein marker (which would indicate current bleeding).

The proposal has also been made that a long tube be passed orally, and fluid aspirated at various levels until blood was found. All tubes have a 'dead space,' which means that large quantities of material have to be evacuated before any appears at the upper end of the tube; in addition, the tube is subject to curling and other difficulties encountered with conventional alimentary tubes.

Two techniques employing radioisotopes have been discussed as possible diagnostic tools in localizing a bleeding site in the gut.

1. Micro-Geiger tubes have been developed (2 mm diameter, 8 mm length), which can be attached to thin wires and swallowed. Beta rays with an energy greater than 0.25 MEV activate the tube. Hence, if red blood cells labeled with radiophosphorus ($P^{32}$, 1.7 MEV beta emission) leak into the vicinity of the counter, radioactivity in excess of the usual background will be recorded.

2. At the time of operation, arteries supplying the viscera can be injected with radioactive materials, and the gut monitored until radioactivity appears in hemorrhagic material within the lumen.

From this, the responsible artery, and often the exact site of bleeding, can be ascertained.[89]

The detection of alimentary hemorrhage, when large, usually presents no problem, since there is gross blood (altered or unaltered) in the stool. As a sequel, the hemoglobin falls (or more reliably in some cases, measurements of the blood volume show a contracted volume). The detection of small hemorrhages into the lumen is usually approached by testing the stool for 'occult blood.' We immediately run into the question: how much occult blood normally enters the stool? It is known, for instance, that after eating rare meat there often occurs a false test for blood in the stool. Certain medications, including aspirin,[58] result in microscopic gastrointestinal bleeding; the ingestion of various dietary items can produce micro-trauma to the gut wall with the appearance of blood in the lumen. Indeed, it appears that 2 ml of blood are lost from the gut hollow each day.[59] The conventional method has been to apply the benzidine,[62,59] orthotolidine,[60] or guaiac[61] tests to stool. The benzidine test depends upon the presence of metallo-porphyrins in red cells. In the presence of a suitable hydrogen donor (such as benzidine), the metallo-porphyrins catalyze ('peroxidatic reaction') the conversion of hydrogen peroxide to water, and the donor is changed to a colored material

$$XH_2 + H_2O_2 \longrightarrow X + 2\,H_2O$$

The guaiac test has also been widely relied upon, since it is supposed not to give false positive results if the patient has been taking iron.[77] More recently, the intravenous administration of blood tagged with radioactive chromium or iron has been used to quantitate gastrointestinal hemorrhage (by the amount of radioactivity appearing in the stool[64,63,65]—this, of course, neglects blood still in the lumen and not yet passed in the stool). After active bleeding stops, following a blood volume determination by the radiochromium technique, stools may be saved to ascertain if fresh oozing is continuing to take place (the appearance of radioactivity in the stool is evidence of continued blood loss, since the radiochromium is bound to red cells). These procedures assume that radiochromium within the lumen is not reabsorbed; in reality, a small percentage does enter through the mucosal cells.[82] A double labeling technique has been described for estimating the quantity of iron which is reabsorbed after it has entered the lumen through alimentary bleeding.[90]

## EFFECTS OF ALIMENTARY HEMORRHAGE

The presence of blood in the gut lumen is often followed by rapid gastrointestinal transit, as well as fever within 24 hours of a major bleeding episode. The cause of the pyrexia is uncertain,[66] since it does not follow experimental introduction of citrated blood into the stomach.[67] Leukocytosis can also occur after alimentary bleeding, and this phenomenon also does not occur when citrated blood is experimentally placed in the lumen.[67]

Sanguinetti (Arch. argent. de enferm. d. ap. digest y de la nutricion. 9:68, 1933) pointed out the occurrence of azotemia following bleeding into the gut. This observation has been subsequently observed and studied in many clinics. The blood urea nitrogen increases within a few hours of a gastrointestinal hemorrhage, tends to reach a maximum value at about 24 hours, and then returns to normal over a period of two days.[68] The quantity of blood, in the alimentary lumen, necessary to produce a specified increase in the blood nonprotein nitrogen is undoubtedly variable, depending upon such factors as fluid balance, hepatic and renal function, and the length of time the blood remains within the lumen. The degree of azotemia is approximately proportional to the quantity of bleeding per unit time. That is, 250 ml of blood in the stomach on each of four days will cause less of a rise in the blood nonprotein nitrogen than 1,000 ml introduced at once. The claim has been made that the degree of elevation of the nonprotein nitrogen may be of prognostic value.[72]

Breakdown products from intraluminal blood are complex, and ammonia is among them (partially produced by bacterial action on the nitrogenous materials). This ammonia is absorbed into the portal circulation and removed by the liver (used in the manufacture of urea). The damaged liver can not handle ammonia properly, and the blood level rises. This has important clinical applications. Upper gastrointestinal bleeding accompanied by an elevated blood ammonia level is highly suggestive of liver disease and esophageal varices.[54] Since intraluminal bacteria contribute to ammonia generation (whether blood is present or not), the use of nonabsorbed antibiotics has been proposed as an adjunct in therapy, and the blood ammonia level may fall following such treatment.[69] The use of enemas to wash blood from the gut, and hence reduce its decomposition, has also been proposed.[70] This may have particular importance as the colon has been reported to be a major site of ammonia production.[71]

Gastrointestinal bleeding has other implications in the body economy. Cardiac changes can occur, varying from minor electrocardiographic alterations to major infarction;[73] hypotension, diminished blood volume, and anoxia have all been mentioned as possible etiologic factors. Finally, the great loss of iron from the body via alimentary hemorrhage should be mentioned again, as this is the commonest cause of iron deficiency anemia in elderly individuals.

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# 13

# PORTAL CIRCUIT AND ENTEROHEPATIC CIRCULATION

Venous blood from the abdominal viscera passes to the portal circuit (Figure 4) and through the liver, before reaching the inferior vena cava. This means that products of digestion which are absorbed and passed into vessels of the intestinal villi, are subjected to hepatic action prior to attaining the main systemic circulation.

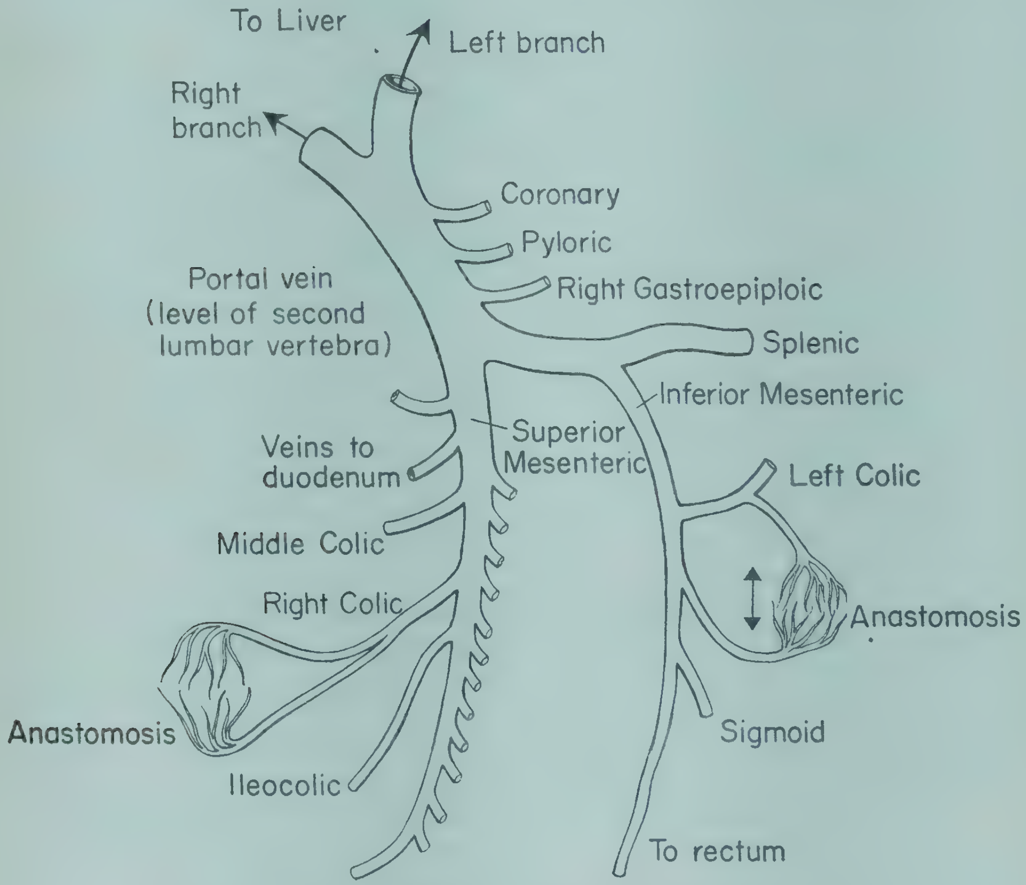
To Liver
Left branch
Right branch
Coronary
Pyloric
Right Gastroepiploic
Portal vein (level of second lumbar vertebra)
Splenic
Inferior Mesenteric
Superior Mesenteric
Veins to duodenum
Left Colic
Middle Colic
Right Colic
Anastomosis
Anastomosis
Sigmoid
Ileocolic
To rectum

Figure 4. Schematic diagram of the portal venous system.

The liver stands between the intestine and the remainder of the milieu interieur. With the exception of lymphatic channels leading from the gut, the portal circuit is the only means of egress for absorbed materials.

Tests of intestinal absorption utilizing moieties taken from the gut lumen and discharged into the blood stream, are intimately tied to the portal circulation and hepatic function. The role of the liver has too often been neglected in the interpretation of absorption studies. For example, the liver clears amino acids unevenly (some more than others) [1] and tests of amino acid absorption are suspect if hepatic function is not clearly defined with respect to the metabolite. The concept of 'clearance,' which has been applied to studies of the kidney (and may be used in evaluating intestinal function, see chapter on Absorption), can be adapted to an expression suitable for following the hepatic uptake of nutrients. The expression is:

$$\text{Clearance} = \frac{(\text{Concentration in hepatic vein})\ \ (\text{Hepatic vein blood flow})}{(\text{Average inflow concentration})}$$

The denominator of the above expression is the average of the concentrations in the hepatic artery and portal vein (both of which supply blood to the liver).

$$\begin{array}{l}\text{Thus:}\\ \text{Average inflow}\\ \text{concentration}\end{array} = \frac{\begin{array}{c}(\text{Hepatic artery conc. x flow}) +\\ (\text{Portal vein conc. x flow})\end{array}}{\begin{array}{c}(\text{Sum of hepatic artery and}\\ \text{portal vein blood flow})\end{array}}$$

For example, if the concentration of an absorbed material was 5 mg per 100 ml in the portal vein when the flow was 300 ml per minute, and 2 mg per 100 ml in the hepatic artery with a flow of 200 ml per minute, the average inflow concentration would be:

$$\frac{(5 \text{ x } 300) + (2 \text{ x } 200)}{(300 + 200)}, \text{ or } 3.8 \text{ mg per } 100 \text{ ml.}$$

The importance of this clearance concept is that its use would separate hepatic activity from intestinal function during experimental tests of absorption.

Abnormalities of the portal and hepatic circulations (Figure

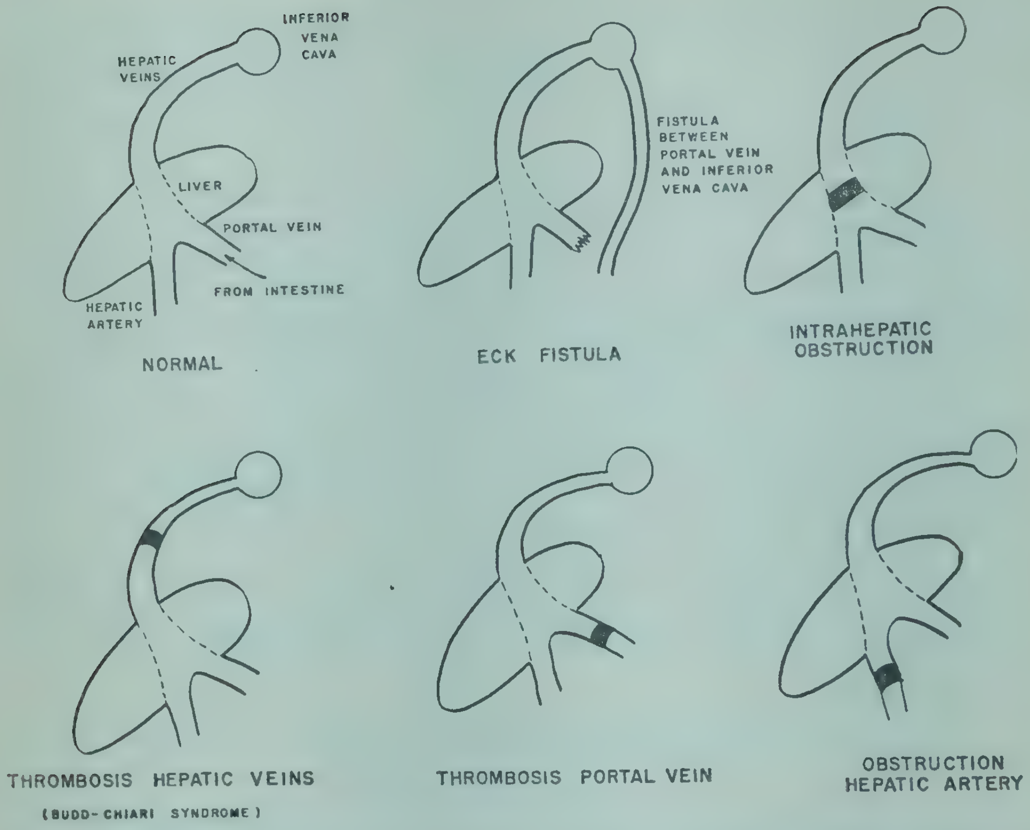
INFERIOR VENA CAVA
HEPATIC VEINS
LIVER
PORTAL VEIN
FROM INTESTINE
HEPATIC ARTERY
NORMAL
FISTULA BETWEEN PORTAL VEIN AND INFERIOR VENA CAVA
ECK FISTULA
INTRAHEPATIC OBSTRUCTION
THROMBOSIS HEPATIC VEINS
(BUDD-CHIARI SYNDROME)
THROMBOSIS PORTAL VEIN
OBSTRUCTION HEPATIC ARTERY

Figure 5. Major vascular abnormalities of the portal and hepatic circulations. (Courtesy of the Navy Medical Department.)

5) are of great clinical importance, and may seriously affect intestinal function. Sudden obstruction of the portal vein produces an increased venous pressure that is reflected back into vessels of the gastrointestinal mucosa,[2] whereas gradual occlusion produces no evidence of mucosal engorgement (perhaps due to development of collateral circuits). Portal obstruction means that absorbed nutrients can not pass readily through the liver, and the systemic circulation is given materials which otherwise would be altered by hepatic action. Because of the importance of these deranged vascular patterns, a brief description is given below; appropriate text books should be consulted for more detailed descriptions.

### 1. Eck Fistula

An Eck Fistula (N. V. Eck, Voyeno Med. J., 1877) consists of ligation of the portal vein, and anastomosis of the proximal end to the inferior vena cava. A significant segment of blood supplied to the liver comes from the portal vein; the Eck fistula results in a diminished blood flow, and changes in hepatic function can follow. This operative procedure passed out of the laboratory stage when it was used in patients suffering from obstruction of blood flow through the liver. An Eck fistula may result in neurologic signs and symptoms following a protein meal ("meat intoxication") and the syndrome is described in detail later. A variant of the Eck procedure is the 'double shunt'; in addition to suturing the proximal end of the severed portal vein to the vena cava, the distal (liver) end is also joined to the vena cava in an attempt to decompress the liver.[3]

### 2. Intrahepatic obstruction

Intrahepatic obstruction most frequently arises from portal cirrhosis. Offering an alternate route about the blocked path, sites of anastomosis between the portal and systemic circuits become dilated (Figure 6). There may be three major clinical manifesta-

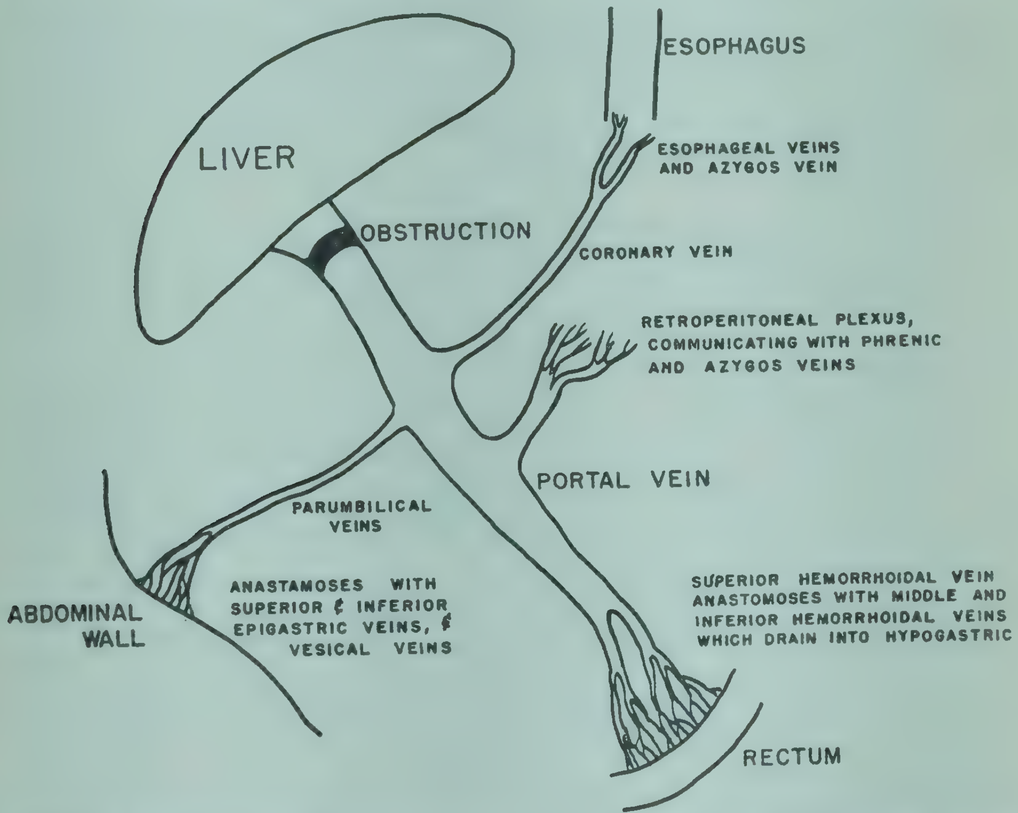
ESOPHAGUS
LIVER
ESOPHAGEAL VEINS AND AZYGOS VEIN
OBSTRUCTION
CORONARY VEIN
RETROPERITONEAL PLEXUS, COMMUNICATING WITH PHRENIC AND AZYGOS VEINS
PORTAL VEIN
PARUMBILICAL VEINS
ABDOMINAL WALL
ANASTAMOSES WITH SUPERIOR ¢ INFERIOR EPIGASTRIC VEINS, ¢ VESICAL VEINS
SUPERIOR HEMORRHOIDAL VEIN ANASTOMOSES WITH MIDDLE AND INFERIOR HEMORRHOIDAL VEINS WHICH DRAIN INTO HYPOGASTRIC
RECTUM

Figure 6. Principal collateral circulation of the portal vein. (Courtesy of the Navy Medical Department.)

tions of the alternate pathways (in addition to generally asymptomatic retrohepatic venous enlargement).

### a. Esophageal varices

These are anastomoses between the coronary vein and esophageal veins. The most frequent cause of dilatation is portal cirrhosis, and bleeding from esophageal varices is a common ending to the disease.

### b. Caput Medusae

This term refers to the dilated 'Medusa head' veins which spread out from the umbilicus in snake-like fashion. Parumbilical veins, descending the ligamentum teres, anastomose with epigastric and vesical veins. Compared with the occurrence of esophageal varices, the Caput Medusae is rare.

### c. Dilatation of Hemorrhoidal Veins

Such enlargement of the hemorrhoidal vessels is not to be confused with the more common 'benign' dilatation and thrombosis. When used as a link between the portal and systemic circulations, the hemorrhoidal veins are large and competent; their lack of valves contributes to the dilatation.

## 3. Obstruction of the Hepatic Artery

Only part of the hepatic blood supply arrives via the artery (the remainder is carried through the portal vein), but a large fraction of oxygen to the liver is carried in the arterial blood. Ligation of the artery may be followed by hepatic necrosis and death. Both frank anoxia and bacterial invasion have been implicated in the mechanism responsible for liver degeneration following hepatic artery obstruction (and antibiotic administration may lead to a more favorable prognosis).

## 4. Obstruction of the Hepatic Veins (Budd-Chiari Syndrome)

This is a rare and dramatic condition[3,4] which may be gradual or sudden. When sudden, there is pain, enlargement of the liver, ascites, and dilatation of the collateral venous system. Splenomegaly and hematemesis are stated to be rare, and this may distinguish the condition from portal thrombosis. An unusual form of the

Budd-Chiari syndrome is found in the West Indies and has been tentatively related to plant toxins (senecio[3]).

### 5. Portal Thrombosis

The most frequent causes of portal vein thrombosis are hepatic cirrhosis, polycythemia vera, infections, and metastatic invasion. Principal manifestations are pain, ascites, splenomegaly and hematemesis.

The portal circuit is difficult to study during life, but evaluation of hepatic function and catheterization of the hepatic vein[5] gives indirect data. Materials may be introduced intrasplenically (radiopaque substances or radioactive tracers) to determine flow through the portal circuit. Using intrasplenic manometry, Davis and coworkers[6] noted that the portal venous pressure, and its reflection in esophageal varices, fell after the intravenous administration of posterior pituitary extract. The suggestion was made that the hormone caused a redistribution of blood flow. At operation, the portal vein may be cannulated to determine its pressure (about 10 mm Hg). Experimentally, the portal circuit has been studied by internal calorimetry[7] and by dye injection. Collaterals of the portal circuit have received clinical attention when dilated, and surgical ligation of varices, bypass operations, and use of the esophageal tamponade have gained acceptance in therapy.

## ENTEROHEPATIC CIRCULATION

By enterohepatic circulation is meant the pathway: absorption from the intestine, entrance into the portal vein, uptake by the liver, secretion into bile, and reentry to the gut (Figure 7). As far as is known, no substance satisfies the above five requirements without entering the lymphatics or at least fractionally passing into the general hemic circulation. There are, however, a number of materials which are partially limited to the enterohepatic circulation: bile salts, iodine and thyroid hormone,[16,17] vitamin $B_{12}$, water, ammonia, and in a restricted sense, cholesterol (and its breakdown products[8]).

It is obvious that substances which are handled in the enterohepatic circulation are not suitable for studies of absorption from the intestine, since their blood levels may have little relationship

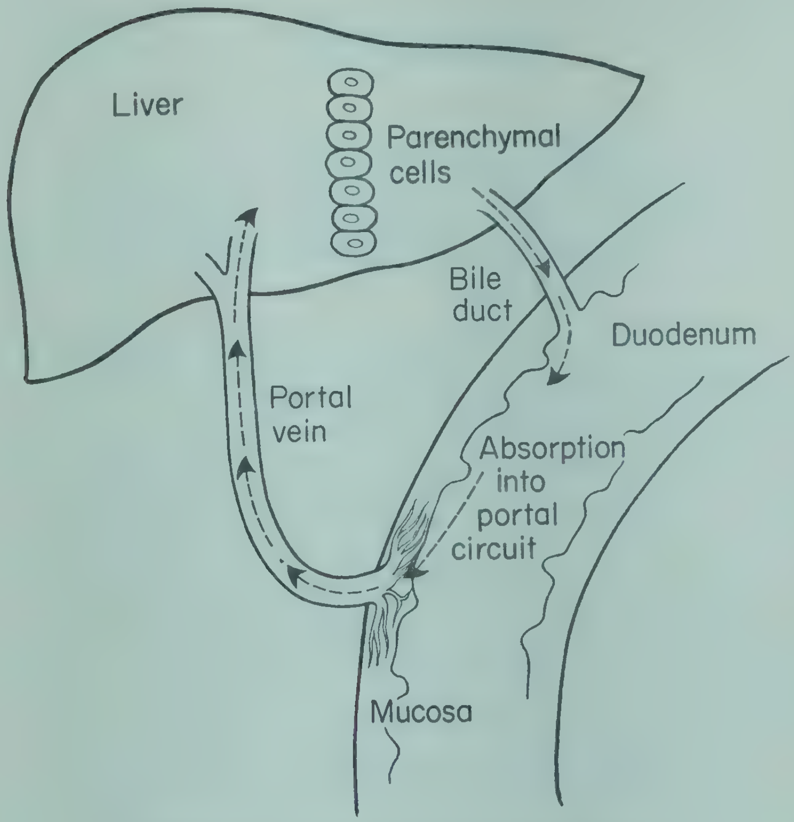

Figure 7. The enterohepatic circulation.

to the quantity extracted from the lumen. A necessary sequel to this pathway is the entrance of materials, usually limited to the enterohepatic route, into the systemic circulation when there is obstruction to biliary outflow; the appearance of bile pigments in the blood stream during obstructive jaundice is the most generally recognized example (but probably not the only one). Continued functioning of the enterohepatic path depends upon the ability of the liver to extract materials from the inflow tract and discharge them into bile (neglecting alterations due to the gall bladder). This extracting ability is interesting from two aspects.

a. Only a relatively minute portion of the materials available to the liver reenter the enterohepatic circulation by secretion into bile. For example, if 5 per cent of the cardiac output of 2,500 ml of plasma per minute entered the liver, $1.8 \times 10^5$ ml of water would pass through per day. Of this, only 500 ml, or 0.28 per cent enters the bile, while 99.72 per cent of the available water is returned to the body.

b. The extracting ability is not the same for all materials. That is, relative to water extracted, more or less of a specific chemical moiety may be taken from plasma and excreted into the bile.

Iodine is another component of the enterohepatic circuit, but is also excreted and reabsorbed by the intestinal wall (see chapter on Hormones). Vitamin $B_{12}$ enters the enterohepatic pathway, but it too is handled by the mucosa. Cholesterol appears at first glance to be handled enterohepatically, but only partially travels this way. During absorption from the gut, cholesterol enters the lymphatics, not the portal circuit, and hence does not initially pass to the liver. The route of cholesterol from gut to lymphatics and thence the blood stream and liver has been termed the 'enterolymphohemohepatic' pathway.[9] As an added complicating factor, the intestinal wall can secrete cholesterol into the lumen.

Ammonia is another material partially handled by the enterohepatic circulation, but in a unique way. Information on ammonia absorption from the gut evolved from studies on animals subjected to an Eck fistula. It was shown (Hahn et al, Arch. d. sc. biol. 1:400, 1892) that dogs so operated upon could not tolerate a diet rich in proteins. A reproducible syndrome was produced when meat was fed, and hence the term 'meat intoxication' gained access to the literature. Symptoms in dogs (irritability, staggering and slapping gait) worsened upon continual feeding of meat, and passed on to coma, occasional convulsions and even death. One of the most striking observations was that withholding meat was generally followed by recovery, and a symptomless prolonged survival.

Protein breakdown products, including ammonia, were investigated as possible causes of the syndrome. Administration of ammonium compounds brought on symptoms of meat intoxication in dogs with the Eck fistula. It was recognized that ammonia entered the portal circuit during meals[10] and that bacteria were partly responsible for the presence of $NH_3$ in the gut. The later development by Conway[11] of a simple means of estimating ammonia in biologic specimens, such as blood, provided a stimulus to further work on the problem of meat intoxication. During the 1930's it was shown that compounds of ammonia, in quantities easily handled by normal patients, produced 'hepatic coma' in patients with severe liver disease. It should be realized that hepatic pathways leading from ammonia to urea can be deranged not only by liver disease itself, but also by vascular abnormalities which prevent blood from adequately reaching the liver (and hence the development of ammonia intolerance in animals after the establishment of an Eck fistula). This point was well proved when it was demonstrated in a human patient with such a fistula.[12] The genesis of ammonia intolerance, and methods for its treatment (including the use of antibiotics to reduce the bacterial population of the intestine) have been summarized in a number of clinical articles.[13,14] An interesting aspect of the genesis of ammonia intoxication is the role played by urea. After production in the liver, the majority of urea is excreted in the urine, but some passes into the gut. Here it may be broken down, by bacterial urease, to ammonia and carbon dioxide. Ammonia is absorbed into the portal circuit, reprocessed into urea, and (aside from dilution) undergoes the cycle again.[15] Hence, a fraction of body urea is handled via the enterohepatic circulation, partly as urea and partly as ammonia. This fate of urea could not be detected easily by the use of the compound labeled with heavy nitrogen (since the ammonia is recirculated and does not readily leave the body). However, it can be noted by employing urea tagged with $C^{14}$. As urea is degraded by intestinal bacteria, labeled $C^{14}O_2$ passes out of the body and can be counted. Such experiments established that bacteria are indeed responsible for the breakdown of urea (this process is inhibited by antibiotics)[15] and also pointed out that mere labeling of a metabolite did not insure its pathways could be easily followed.

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# 14

# HORMONES

THE INTESTINAL tract is in the unique position of being affected by two groups of hormones—those of the major endocrine glands, and those of the alimentary canal itself. As far as known, there is no hormone produced outside of the gastrointestinal tract whose primary locus of action is the gut. Thus, hormone activity upon the digestive tract must be viewed as but one effect among many. The specific mechanisms whereby hormones influence the gut are uncertain, although a beginning has been made in understanding the adaptation of enzymes, those within the intestine included, to changing hormone levels.[1]

## PITUITARY HORMONES

Principal effects of pituitary trophic hormones on the intestine are mediated via their action in liberating other endocrine substances from target endocrine organs. That is to say, the adrenocorticotrophic hormone influences the intestine primarily through the release of adrenal hormones which have an intestinal effect. Hypophysectomy is followed by profound changes in the mucosa and smooth muscle of the intestine,[2] probably due to panhormone deficiency. In addition, the intestinal level of secretin falls after hypophysectomy.[112] There are also a few known reactions in which pituitary secretions directly influence the gut.

### POSTERIOR PITUITARY

The posterior pituitary produces two hormones—a vasoactive principle and an oxytocic substance.[2] Both have been studied for effects on the intestine.

The vasopressor hormone does not delay the absorption of water from the human intestine;[4] it has been reported to actually increase the rate of water uptake from isosmotic saline placed in the small intestine of animals.[5] Removal of vasopressin from the circulation is not a function of the small intestine,[6] although the hormone has a pronounced effect on the splanchnic circulation. The pituitary vasopressor substance depresses portal vein pressure in animals,[7] lowers the pressure of the portal system when administered during cirrhosis of the liver in man,[8] and has been used as an adjunct in the treatment of bleeding from esophageal varices.[9] The cause of this circulatory change due to vasopressin is not clear, although blood may be drained from the splanchnic area.[8]

Studies of the vasopressor and oxytocic fractions showed both to affect the gut[10,11,12] (the pressor hormone being more active than the oxytocic principle). Species differences existed as to whether the small intestine or the colon was maximally acted upon, and the circular and longitudinal muscle groups did not react in the same manner (see section on Metabolism of Intestinal Muscles). Whole posterior pituitary extract diminishes the tone and movement of the human stomach according to Grollman.[13] In enormous doses, the hormones may cause ulceration of the gastric mucosa (which is probably a toxic effect and not of physiologic significance[146]). Movement of the small gut and colon, initiated by the pituitary vasopressor hormone,[15] begins within a few minutes and is rapidly dissipated in human beings.[14] The net effect of whole posterior pituitary extract is stimulation of peristalsis with little change in tone.[15] The pressor hormone has enjoyed some use in the treatment of postoperative distention and the clearance of air from the bowel prior to x-ray studies. Parasympathetic medications, however, can perform the same task without the dangers involved in using a strongly pressor substance.

## ANTERIOR PITUITARY

### Thyroid Stimulating Hormone (TSH)

Injected exogenous thyroid stimulating hormone could be recovered in greater amounts if animals were first subjected to evisceration-hepatectomy.[16] The poor condition of the animals so treated precluded definite conclusions as to the role of the gut in TSH inactivation. The majority of the effects of TSH on the intestinal tract appear to be mediated through the liberation of thyroid hormone (q.v.).

### Growth Hormone

Effects of the growth hormone are difficult to separate from that of other pituitary secretions, and intestinal changes following hypophysectomy[2] are undoubtedly due to a pan-hormone deficit. Selye[17] states that enlargement of the viscera during the course of pituitary gigantism remains proportionate to the corresponding size of the body, while in acromegaly (hypersecretion of the growth hormone during adult life) the viscera are more than correspondingly enlarged. Acromegaly has also been rarely associated with ulcers of the small intestine.[143] A group of French workers reported that administration of growth hormone resulted in correction of the deficiency syndrome which follows gastrectomy,[18] but this has not been confirmed.

## EPINEPHRINE - NOREPINEPHRINE

These hormones of the adrenal medulla are poorly absorbed from the gastrointestinal tract. When given by any of the parenteral routes, striking changes are engendered in the gut and its blood supply. Many capillaries in the mesentery become constricted,[19] so that blood flows through relatively few channels. There is diminution or frank cessation of movement of the stomach and intestines following administration of large doses of epinephrine. Sensitivity of the alimentary canal to this hormone was recognized early and shown by bioassay.[144] In addition to the inhibition of intestinal movement, epinephrine tends to exert a tonic action on the sphincters, so that the net effect is constipative.

Effects of epinephrine on the gut probably arise in vivo from adrenergic nerves (even on prolonged stimulation of adrenergic nerves to the gut, almost no epinephrine is liberated into the blood stream [20]). It is probable that adrenergic action on the gut is the sum of the effects of epinephrine and its analogue norepinephrine (which differs in having the terminal methyl group replaced by hydrogen). Epinephrine and norepinephrine are also found in chromaffin tissue[21] located in the gut or in other locations (see chapter on Carcinoid Syndrome). The action of adrenal medullary hormones on small gut secretions[22] are perhaps mediated through effects on altered motility and blood supply, but further work is needed. Epinephrine derivatives have varying degrees of potency against the intestine, and some even show a stimulation of tonus rather than an inhibition.[23] Epinephrine has been noted to alleviate acute allergic symptoms in the intestine.[147]

## ADRENAL CORTICAL HORMONES

An enormous literature has sprung up about the adrenal cortical hormones and their effects on the alimentary canal (particularly the relationship to peptic ulcer and uropepsin secretion). A summary article by Zetzel[24] may be consulted for clinical aspects of the use and complications of these hormones in diseases of the digestive system.

Adrenal steroids are absorbed when given orally, and a small percentage appears in the stool following administration of cortisol[25] or hydrocortisone.[26] The precise route of absorption is uncertain. When given rectally, absorption may not take place;[28] cortisol has been noted to be absorbed from the rectum,[148] an event inhibited by neomycin. Despite the catabolic effects of the adrenal steroids, gastrointestinal anastomoses appear to heal well during administration of adrenocorticotrophic hormone.[27] Addison's disease (severe adrenal hypofunction) may be marked by alimentary symptoms (nausea, hiccups, change of bowel habits, abdominal pains) and a diminution of secretions. At the other extreme, morphologic changes in the gut and overt symptoms during adrenal hyperfunctioning (Cushing's disease) are not well defined. Administration of exogenous steroids, however, may be complicated by ulcerations and perforation of the small bowel.[30]

The precise mechanism by which adrenal hormones produce their effects in the intestine has not been elucidated; more than one pathway may be operative. Several enzymes have been studied in the gut during adrenal steroid administration or deprivation, but no consistent pattern has emerged. Following adrenalectomy, intestinal histaminase decreases in the cat.[31] Adrenal steroids enhance the action of intestinal transaminases.[33] Adrenalectomy did not affect the acid or alkaline phosphatase levels of the gut of male rats,[34] but administration of desoxycorticosterone acetate to young rats increased intestinal alkaline phosphatase.[29]

Small doses of adrenal steroids increase gut motility,[35] and these hormones also exert an effect on electrolyte exchange across the intestinal wall. Adrenalectomy decreases the rate of alimentary absorption of sodium, and to a lesser degree, of potassium.[36,37] Desoxycorticosterone, when administered to patients receiving a cation exchange resin, decreased fecal sodium loss, while increasing the output of potassium.[38,39] Synthetic fluorohydrocortisone has a similar effect,[40] indicating that the steroids may play a somewhat identical role in the gut and kidney. Ability to alter the osmolarity of intraluminal fluids may be diminished following adrenalectomy,[36] and there can be absorptive errors as well.[41] Information on the ability of the steroids to stimulate intraluminal enzymes and their inhibitors is fragmentary.[42] Three recent reports shed additional light on the adrenal-gut interrelationship. Aldosterone produces a fall in the fecal Na/K ratio,[151] cortisone decreases the ability of the gut to conjugate o-aminophenol,[152] while adrenalectomy is without effect on the intestinal content of citrate.[153]

## HISTAMINE

It is difficult to place histamine in any single classification, since it is probably present in all tissues in bound form. We are considering it in the section on hormones because it is a known powerful stimulant to gastric acid secretion and has other effects on the gut, such as contracting intestinal musculature. Histamine is detectable in the stomach and small intestine of many animal species as well as man.[43,45] Its function in these locations is uncertain. There is no clear evidence that histamine is the naturally occurring gastric hormone, and it has been claimed that a protein fraction can be separated from the pylorus which stimulates hydrochloric acid secretion, without the presence of histamine.[44]

The enzyme histaminase, capable of degrading histamine, can be demonstrated in the small intestine.[46] Since intestinal bacteria produce histamine from the amino acid histidine, histaminase may teleologically be viewed as a defense against the entrance of intraluminal histamine into the body. Only minute amounts of histamine exist within the alimentary contents.[47] Even when large quantities of histamine are introduced into the gut, only a minor fraction can be recovered from the intraluminal material.[48] Large amounts of histamine can be tolerated when taken orally;[49] the protective effect against histamine is contained in the gut wall and injuring the mucosa by detergents allows histamine to enter.[50] The intestine can be a target organ in an anaphylactoid response,[52] but the exact role of histamine in the process is unknown. The action of histamine on the gut is partially antagonized by acetylcholine,[51] a process whose physiologic significance has still to be defined.

## ACETYLCHOLINE

The principal effects of acetylcholine on the gut are a stimulation of smooth muscle contraction, and a stimulation of secretion (this is particularly marked in the stomach). These actions are mimicked by acetylcholine-like drugs[53] and antagonized by atropine and its analogues.[54] A number of acetylcholine derivatives have been studied, since they are not as easily hydrolyzed (and hence inactivated) as the parent compound; they have been stated to be useful in overcoming postoperative distention and in other special situations.[56,57] The effect of the vagus nerve on the intestine is probably mediated through acetylcholine or similar molecules.

The ability of atropine, an anticholinergic, to stop the flow of intestinal secretions produced by vagal stimulation was recognized over 40 years ago (Savich, V. V.; Soshestvensky, N. A.: Compt. Rend. Soc. Biol. 80:508, 1917). Anticholinergics may also alter absorption,[58] but how much of this is due to an antagonism of acetylcholine is unknown.

Medications which either interfere with acetylcholine degredation (by inhibiting cholinesterase)[55] or represent acetylcholine analogues not readily split, can cause severe gastrointestinal symptoms, due to hypermotility and hypersecretion. In the extreme case, parasympathetic medications produce a bloody diarrhea in dogs and a syndrome similar to ulcerative colitis.[59]

Acetylcholine normally occurs in the wall of the alimentary canal,[60] and a larger amount can be detected after a segment has been stretched. There is a report that cocaine does not inhibit acetylcholine production by intestinal strips in vitro,[61] and the process may thus be somewhat independent of intrinsic nerves. Calculations of the amount of acetylcholine in specific regions of the intestinal wall must take into account the fact that more than one site probably manufactures the hormone.[62,63]

## INSULIN

The action of insulin on the intestinal tract is complex, involving changes from hypoglycemia and perhaps alterations due either to the hormone itself or to its secondary manifestations. Bachrach[64] has summarized much of the pertinent literature. Most work has been in connection with the increased outpouring of gastric acid during hypoglycemia, and the use of this procedure in testing for the completeness of surgical vagotomy (the acid response to hypoglycemia is diminished following vagotomy[65]).

In alloxan diabetes, conversion of carotenes to active vitamin A by the intestinal wall is severely reduced, and may be the reason for the occasional finding of diabetic carotenemia.[66] Unusual and disturbing complications of diabetes mellitus which affect the gut are diarrhea[67] and steatorrhea.[68,69,70] Involvement of the nervous system is a common occurrence in diabetes, and it is apparently this alteration which results in aberrant intestinal motility and changes in absorption.[70]

An interesting syndrome of gastric hypersecretion, peptic ulceration and islet-cell tumor of the pancreas has been recognized clinically.[71] The tumors do not produce insulin, and their mechanism of action has still to be defined.

## PARATHYROID HORMONE

The parathyroid glands are involved in the complex mechanism which regulates body calcium levels. The major portion of dietary calcium is not absorbed and passes out in the stool; part of the excreted calcium arises from endogenous' calcium which has been secreted into the gut (see chapter on Absorption). Parathyroid hormone may have a stimulatory effect on calcium uptake from the gut lumen[145] (results of such experiments are difficult to interpret since the hypocalcemic gut may be subject to tetany and functional abnormalities). Vitamin D and dihydrotachysterol are more effective in enhancing calcium uptake.[72] Parathyroid hormone is protein in makeup, and ineffective if taken orally. Parenterally administered parathormone has been shown to influence intestinal clearance of various metabolites from the blood.[144]

The influence of the parathyroid glands on other intestinal functions is not clear. Hypoparathyroidism is manifested by tetany which can affect the gastrointestinal musculature throughout its length,[73] producing spastic contractions, pain and vomiting. Parathyroidectomy has been reported to increase trypsin and chymotrypsin secretion in dogs and to augment gastric hydrochloric acid secretion.[74] This seems to be diametrically opposed to observations that hyperparathyroidism in man is accompanied by a high incidence of peptic ulceration[75] (other alimentary symptoms may also be present [76]) . The parathyroids appear to be normal in cases of functional alimentary distress.[77]

Patients with peptic ulceration often rely on milk and alkali for relief of their symptoms. This combination of high calcium intake and the presence of excess alkali results in a large net calcium absorption, as well as renal damage due to calcium deposits. A chemical picture resembling hyperparathyroidism can result, and has been termed the milk-alkali syndrome.[78] Features of the syndrome which enable the diagnosis to be established are the history, and the finding of an elevated blood calcium without a corresponding increase in the urinary calcium output.

Just the opposite sort of picture (decreased calcium uptake) can also be encountered clinically. In cases of steatorrhea, fat lost in the stool carries calcium with it (calcium soaps),[79] and lowers the blood calcium level by reducing the amount which passes into the body. In severe cases of malabsorption, blood calcium may be low enough to result in clinical tetany. Occasionally, cases of disabling skeletal pain have been reported due to 'occult' steatorrhea and calcium loss.[80] Calcium absorption may also be reduced by using substances which chelate calcium within the lumen and render it less available for uptake. Such a chelating material has been tried in individuals with urinary calculi,[81] and in patients on prolonged bed rest who show hypercalcuria due to bone resorption.[82] Sodium phytate (inositol hexaphosphoric acid in the form of its sodium salt) blocks calcium absorption. When taken orally, sodium phytate combines with calcium, producing salts which are insoluble or poorly dissociated.[82]

Blood calcium levels may also be affected in other gastrointestinal disorders without clear evidence that the parathyroid glands are at fault. During bouts of pancreatitis, pancreatic enzymes digest tissues in the region of the pancreas, and calcium combines with breakdown products (particularly fatty acids) to lower the blood level. Hypocalcemia has also been reported in a few cases of small bowel infarction[83] and perforated peptic ulcer.[84]

## ANDROGENS

Testosterone is slightly absorbed from the alimentary canal, and its methyl derivative is taken up in larger quantities (especially when the sublingual route is used). About 14 per cent of 4-$C^{14}$-testosterone appears in bile, primarily in conjugated form.[85] However, less than this amount eventually comes out in the stool (and that which does is not conjugated), suggesting breakdown of the conjugate in the lower intestine. Effects of androgens on the intestine are not pronounced, and hypogonadism in the male is not particularly associated with morphologic changes in the alimentary canal. Cappelli and Tacconi,[86] in an unconfirmed report, state that testosterone did not affect the quantity of water absorbed by male castrate rats, but increased water uptake in female castrates. Cappelli and Rossi[87] reported water absorption from the intestine fell in adrenalectomized rats and that the defect was not corrected by testosterone in male rats; however, testosterone was beneficial in female adrenalectomized rats.

## ESTROGENS

The estrogenic steroids and related synthetic materials are effective when taken orally. A large fraction of labeled progesterone appears in the bile following parenteral administration,[88] suggesting that this is a normal excretory pathway. Alteration of the chemical structure of estrogens occurs in the feces,[89] perhaps due to bacterial action.

Estrogens appear to have little effect on the intestine, except when given in very large doses (such as during the treatment of malignancies [90]) . Oophorectomy is followed by atrophic changes in the oral mucous membranes, but the remainder of the digestive tract appears normal. Neither estradiol nor progesterone corrects the deficit in intestinal water absorption caused by adrenalectomy in the rat.[87] Progesterone is claimed to be less effective than estradiol in correcting defective water absorption after castration.[86] Interestingly, one of the routes of estrogen excretion by the newborn is via the feces.[150] During pregnancy, pregnanediol appears in the feces.[154] The alimentary loss of this steroid has been thought to explain the variable quantity appearing in the urine. A stimulatory effect on galactose metabolism in the gut, by both male and female sex steroids, has been noted.[155]

Whether a component of the cramps associated with dysmenorrhea is due to gastrointestinal action is uncertain. In the premenstrual period there may be abdominal pains, a sensation of a need to defecate, and the expulsion of scybalum. It is known that, premenstrually, women retain water; this is a possible explanation for dryness of the fecal mass, with excess water having been taken up from the gut.

The corpus luteum produces, in addition to progesterone, a hormone termed relaxin. No information appears to be available on the effect of this agent on the gut, although it has been used in the treatment of radiation stricture of the bile duct.[91]

## THYROID HORMONE

Ingested iodine-organic complexes are absorbed from the alimentary canal, but probably at a slower rate than iodine itself (which is taken up in the form of iodide) . In hypothyroid subjects, the rate of iodine absorption is slower than normal. Thyroxin and triiodothyronine are likely absorbed from the lumen without alteration. Thyroxine can be found in the intestinal contents and feces, probably reaching these sites via biliary excretion.[92,93,94,95] Iodine also occurs in the alimentary lumen and feces, being excreted by a gastric mechanism,[96] passing through the intestinal wall,[97] and likely passing out in bile. At least in certain animals, the intestine can produce a thyroxin-like protein when iodine is present,[98] but the magnitude of this contribution is small.

The quantity of ingested thyroxin eliminated by the intestinal tract is dose dependent. When large amounts are taken orally, almost all may be recovered in the feces.[92] Most thyroxin excreted in normal bile is in the form of a conjugate (perhaps with glucuronic acid [94]). Liver and kidney slices seem able to produce this conjugate, but the material can be subsequently broken down in the intestine so that free thyroxine appears in the stool. Loss of thyroid hormone in feces was teleologically claimed to serve no purpose. It was considered that the enterohepatic circulation rechanneled most of the hormone,[99] and that no more than a small fraction appeared in the stool.[100] However, more recent data on human subjects [101,102] indicate that biliary clearance of thyroxin is equal to 200 to 600 ml of plasma per day, and that more than half of this is lost in the feces.

### Signs and Symptoms

One of the early and persistent symptoms of hyperthyroidism is an increase in bowel activity; the converse, decreased activity and constipation in hypothyroidism, has also been noted. Emesis has uncommonly been reported in cases of hyperthyroidism. Abdominal pains may occur in thyrotoxic individuals and in a number of these cases, hypercalcemia is present.[111] Upon treatment of the thyroid overactivity, the pains disappear as the blood calcium level returns to the normal range. A common alimentary finding in thyroid disease is achlorhydria, and this may be found in both hypo- and hyperthyroidism.

### Motility

Motility of the dog's stomach is increased by the feeding of thyroid;[103] contractions induced by use of the hormone can be partially decreased by vagotomy.[104] Although there is some difference in results obtained,[105] gastric emptying of carbohydrates is perhaps more rapid than normal in thyrotoxic rats[106] and human beings.[107] Measured by the passage of barium through the small gut into the cecum, transit time is accelerated in thyrotoxicosis.[105, 108] The tone of the colon has also been reported to be increased in this disorder,[105] and the roentgenographic pattern of the small bowel mucosa may be disturbed. Rare cases of diminished absorption in thyrotoxicosis have been noted and the assumption has been that a rapid transit through the gut more than compensated for any absorptive increases.

Diminished intestinal motility might be expected in myxedema; there are several reports of this occurrence,[109,110,113] although documentation by present techniques has not been adequately presented.

### Secretion

Information is meager on quantitative changes in alimentary secretions during thyroid dysfunction. The action of thyroid hormone on the gut is apparently not dependent upon extrinsic nerves.[114] The hyperthyroid dog has been noted to have an increased volume of jejunal secretions as well as an elevated concentration of several enzymes within the fluid.[115] About one-third of human patients with hyperthyroidism have gastric anacidity,[116,117] and achlorhydria is commonly observed in hypothyroidism as well.[116]

Changes in alimentary secretions during hypothyroidism are undoubtedly secondary to morphologic alterations in secretory cells. The alimentary tract can be strikingly affected by far advanced hypothyroidism.[109,118,119] An atrophy of the lining membrane is common, particularly affecting the oral and gastric mucosa. The intestinal mucosa may reveal diminution of glandular crypts and occasional cystic dilatation of the crypts. In advanced myxedema, infiltrates occur throughout the entire bowel wall, producing a thickened and leathery appearance. Distention and elongation of the colon have been reported in myxedema,[109] as well as a megasplanchnia, particularly of the colon and duodenum.[120]

### Absorption

The effect of thyroid hormone on absorption from the intestine has been reviewed by Althausen.[106,121] The hyperthyroid rat has an accelerated uptake of glucose, oleic acid and galactose (but galactose uptake is normal in hyperthyroid human patients [123]). The absorption of xylose, alanine and calcium lactate is unchanged in hyperthyroid rats. An effect of thyroid extract on phosphorylating systems has been proposed to account for changes in absorption;[121] in vitro, thyroxin increases the quantity of energy-rich phosphate compounds within the gut wall.[122] Hyperthyroidism in man may be associated with an elevated blood sugar level, perhaps partly due to rapid glucose uptake from the gut, and to dumping of carbohydrate from the stomach to the intestine.[107] Slowed glucose absorption occurs in rats who have been thyroidectomized; the flat oral tolerance test curves for galactose,[127] and glucose, in hypothyroidism perhaps indicate delayed absorption of these monosaocharides. In addition, the rate of absorption of vitamin A and fats is slower in cretins than in normal individuals,[128,129] and vitamin $B_{12}$ absorption may be diminished.[130] Whether such effects are due to alterations of cellular permeability or changes in specific enzyme systems is unknown.

In hyperthyroid rats the absorption of oleic acid, but not olive oil, was accelerated.[106] If correction is made for the time probably required to split olive oil into its component parts, there may be little difference between the absorption of the fatty acid and the complex oil. In dogs made thyrotoxic, olive oil administration was followed by abnormally high concentrations of fatty acids.[131] However, stools were not collected to determine if more than the normal amount of the material had been absorbed. Some human patients have been reported to lose excess fat in the stool during thyrotoxicosis,[132] especially if diarrhea was present. Perhaps the increased peristalsis and decreased transit time more than counterbalanced any tendency toward accelerated absorption.

In both clinical hyperthyroidism and experimental thyrotoxicosis, increased calcium and phosphorus excretion occurs in the stool,[125] and an increased calcium turnover has been demonstrated.[126] Any explanation for the excess calcium loss must account for the following facts.

1. During thyrotoxicosis, absorption of calcium salts placed in the small intestine appears to be unimpaired.

2. Diminution of fecal calcium output occurs in hyperthyroid rats who have been treated with morphine or given a lessened food intake.

3. On a calcium-free diet, thyrotoxic rats still excrete more calcium than their normal littermates. Excretion of calcium in normal

animals can be increased by subdiarrheal doses of cascara or castor oil.

From such data, Althausen and Stockholm[106] concluded that overeating and hyperperistalsis accounted for the excess loss of calcium in the stool during thyrotoxicosis. Overeating presumably increased the fecal mass, and water within the bolus may have contained calcium. Hyperperistalsis, particularly diarrhea, was viewed as aggravating the calcium loss; steatorrhea also elevates fecal calcium loss by forming calcium-soaps. The extent of calcium absorption in hypothyroidism is not agreed upon,[128,125] and is probably related to the fat content of the diet.

Carotene is converted to vitamin A in the small intestine[124,133] (see discussion of vitamin A) ; the thyroid hormone may be essential for this mechanism. The complex reaction[134] is blocked in alloxan-induced diabetes,[135] perhaps explaining the carotenemia occasionally seen in diabetics (and indicating that the overall process is somehow related to hormone levels) . There may also be a defect in absorbing preformed vitamin A in hypothyroidism.[128,129] Increased fecal carotene excretion has also been noted in experimental hypothyroidism.[136,137] An inability to store vitamin A when fed as carotene[138] may contribute to the development of night blindness in hypothyroidism.

The hyperthyroid animal has an increased metabolic turnover and an elevated requirement for vitamins.[139] Whether vitamin absorption is affected has not been clearly demonstrated; it has been shown that a number of conditions which adversely affect the intestine diminish vitamin uptake.[140] The thyroid does not seem to be specifically related to other intestinal disorders, and has no effect on the healing of experimental ulcers.[141,142]

## PREGNANCY

Pregnancy, which can be viewed as a multiple endocrine syndrome, is associated with a high incidence of certain gastrointestinal disturbances (cholelithiasis, 'indigestion,' constipation, enteroptosis, and the nausea and vomiting of morning sickness) . Intestinal displacement, mechanical pressure of the enlarged uterus on the bowel, and perhaps the relaxant effect of progesterone on smooth muscles, may all contribute to the constipation. Pregnancy has been noted by some clinicians to bring relief from the symptoms of peptic ulcer, and a reduction in gastric acid production during pregnancy has been claimed. However, not all studies have been able to confirm this.[32]

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## 15

# VITAMINS

A DISCUSSION OF vitamins and the intestinal tract would logically include: synthesis and degradation by intestinal bacteria,* absorption from the gut lumen, changes made in the compound within mucosal cells, route of egress, and the effects of hypo- and hypervitaminosis on intestinal function. However, all of these aspects have not been clearly evaluated for each vitamin. For ease of presentation, the vitamins will be considered separately, with cross references when necessary.

## VITAMIN A

Carotenes (alpha, beta, gamma), which occur in plants, and perhaps other precursors of the vitamin,[3] are converted to vitamin $A_1$ by animals. Less commonly, vitamin $A_2$ is obtained by eating fish. Most carotenes are changed, mole for mole, to vitamin A esters. However, by symmetrical fission, beta carotene forms two moles of vitamin A. The conversion of precursors to the vitamin occurs principally in the wall of the small intestine. The conversion is reduced 75% in alloxan diabetes,[1] and may be the reason for diabetic carotenemia. There is also difficulty in carrying out the reaction: carotene $\longrightarrow$ vitamin A in the absence of thyroid hormone.[2] The conversion of carotene to vitamin A alcohol likely involves the intermediate formation of an aldehyde.[63]

In animals, the lower small intestine is a major site of vitamin A uptake.[4] The absorptive mechanism which handles vitamin A is complex.[5] To begin with, the vitamin is insoluble in water; its uptake appears to depend upon the presence of bile[6] or a suitable emulsifying agent. In pancreatic disease, vitamin A alcohol is more readily absorbed than the ester; in celiac disease, absorption of both the alcohol and the ester is diminished.[5] This has been interpreted as meaning that vitamin A esters are split in the intestine and absorbed as the alcohol form. Water soluble derivatives of vitamin A are available, and are better absorbed than the standard fat soluble preparation.[7]

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*Additional aspects of the synthesis and degradation of vitamins are discussed in the chapter on Intestinal Bacteria. Aberrations of vitamin absorption are mentioned in the chapter on Malabsorption.

The absorption of carotene may be schematized as shown in Figure 8.

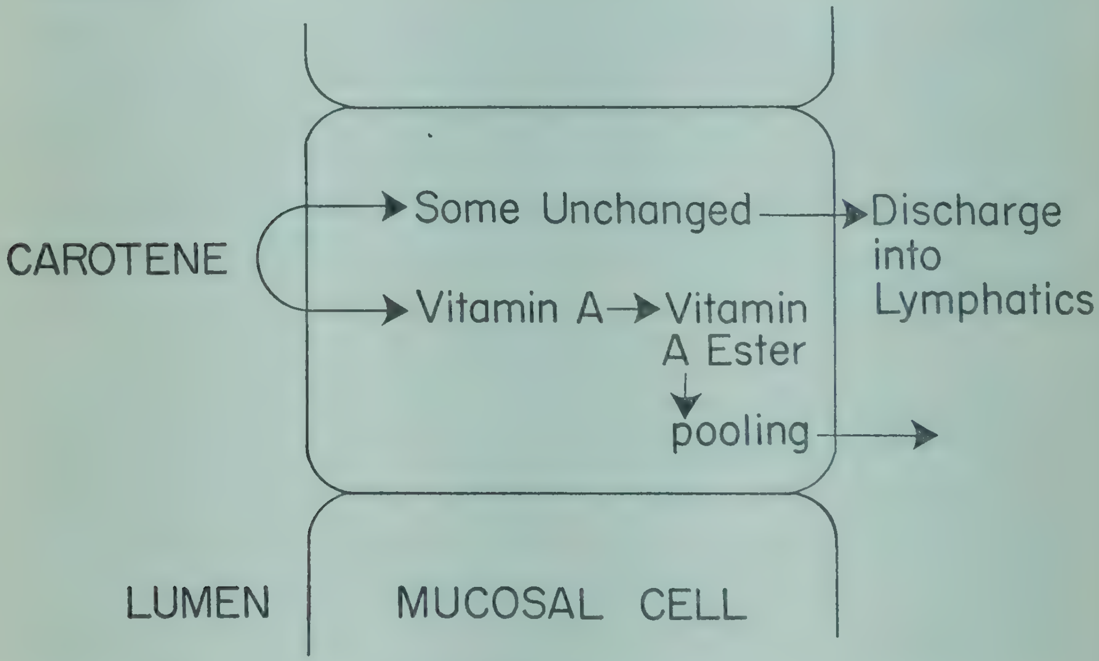


Figure 8. Intestinal absorption of carotene.

It appears that vitamin A must be esterified in the intestinal wall before being discharged into the lymphatic stream.[8] Pooling of the vitamin or its ester in the mucosa can result in a delay in the appearance of ingested vitamin A in the peripheral circulation.[9] Some carotene and vitamin A are destroyed in the alimentary lumen. Soy beans contain an enzyme (lipoxidase) which inactivates carotenes,[10] but whether this occurs within the lumen is unknown. Certain substances have a protective effect on vitamin A and carotene[12] and this action may be operative within the lumen.[11]

Absorption of carotene is interfered with by the presence of mineral oil.[13] Prolonged use of mineral oil is accompanied by a reduction of the blood carotene level with little change in the vitamin A concentration.[14]

Vitamin A absorption has been used as a clinical test of the competence of the absorptive mechanism.[15] However, as we have discussed, the test assumes normal biliary activity is present. The blood carotene level is often reduced in patients with malabsorptive diseases[16] but carotene tolerance tests have not been widely employed. Individuals with gastrointestinal malignancies frequently have reduced blood levels of vitamin A,[17] perhaps partly due to inanition.

Absence of vitamin A from the diet for a prolonged period results in a number of aberrations, including degeneration of columnar and cuboidal epithelium into stratified squamous epithelium. This metaplasia affects the pancreatic and biliary ducts; in rats it produces alterations of the forestomach.[18] Hypervitaminosis A does not appear to primarily affect the gut,[19] and alimentary symptoms are not prominent.

## VITAMIN C

Water soluble vitamin C is absorbed from the small intestine of animals, and may be stored in the intestinal wall.[20] Some species of bacteria in the gut can destroy the vitamin,[21] and decreased uptake of vitamin C in achlorhydria is perhaps partly due to the overgrowth of these microbes. Low excretion of vitamin C has been noted in patients being treated for peptic ulcer, likely resulting from the low ascorbic acid content of the ulcer diet.[22] Other intestinal disorders perhaps also contribute to the development of hypovitaminosis C.[23] In animals, lack of vitamin C has been thought to be a contributing factor to the experimental development of gastric ulcers. It is known that the vitamin is required for tissue repair and the synthesis of collagen (precursors of collagen have been noted in the stomach,[24] but not the small intestine). Ascorbic acid can be detected in the gastric secretion.[25] One of its functions in this locale may be reduction of iron from the ferric to the ferrous form (as only ferrous iron is suitable for absorption).

## VITAMIN D

The mechanism of action of this 'fat soluble' vitamin is uncertain, but may be related to citric acid metabolism.[26] Vitamin D has an important effect on the gastrointestinal canal, where it is more effective than either parathyroid hormone or dihydrotachysterol in facilitating calcium absorption.[27] Enzymes known to have an effect on calcium and phosphorus metabolism have been studied in the gut, but have not yet provided a lead as to the reason for altered calcium intake. For example, the intestinal mucosa contains a number of phosphatases (at least part of which are sensitive to adrenal steroids [28]). There is a report that vitamin D increased the activity of an alkaline phosphatase of the rat intestine;[29] however, intestinal secretions from rachitic dogs revealed no diminution in phosphatase activity.[30] The precise alteration which permits more calcium to be absorbed is thus uncertain (but must be an effect directly on the gut, since it is shown by isolated gut sacs [59]). It is known that phytic acid binds calcium and makes it less available for absorption.[58] According to one report, following administration of vitamin D, an enzyme which cleaves phytates could be recovered from the gut in increased amounts;[31] this may be an important lead, since phytase appears to be concentrated primarily in the intestine.[60]

Hypovitaminosis D (rickets) is marked by failure to absorb sufficient calcium from the intestine; hence alkaline calcium salts appear in the stool. Hypervitaminosis D likely causes its symptoms by an intensification of the normal role of the vitamin.[27] Increased alimentary absorption of calcium leads to an elevated blood calcium level, depression of the parathyroid glands, and virtual cessation of urinary phosphorus excretion. The blood stream is then saturated with calcium and phosphorus, and precipitation occurs at abnormal locales such as the stomach wall. Gastrointestinal symptoms occur during vitamin D intoxication.[32] Overdosage of the vitamin is to be distinguished from the milk-alkali syndrome seen in individuals taking large quantities of milk and alkali in the treatment of peptic ulcer (this syndrome is also accompanied by elevated blood calcium levels).

## VITAMIN E

Vitamin E is another 'fat soluble' accessory food substance, whose precise role in human nutrition is not clearly established, although it may serve as an activator of enzyme systems.[33] Evidence has accumulated that the vitamin possesses antioxidant properties. In the alimentary lumen, vitamin E and other tocopherols prevent oxidative inactivation of carotene.[12] Interestingly, the absorption of tocopherols is stated to be increased in hepatic cirrhosis.[34] It has been reported that gamma tocopherol is well absorbed from the intestine.[64] Low plasma tocopherol levels occur during steatorrhea.[65]

## VITAMIN K

Still another 'fat soluble' factor is vitamin K. It is essential for the formation of prothrombin by the liver. In deficiencies of prothrombin, bleeding may occur—including gastrointestinal hemorrhage. Cases of sprue exhibiting hypoprothrombinemia have been reported, probably due to impaired vitamin K absorption.[35]

Simple (dietary) lack of vitamin K is rare, because of its widespread occurrence and bacterial synthesis. However, a 'conditioned' deficiency of the vitamin can occur in obstructive jaundice (since the 'fat soluble' vitamin is poorly absorbed in the absence of bile[36]). It is possible that even in the face of adequate amounts of vitamin K, liver disease may be severe enough to result in inadequate production of prothrombin.

## THE VITAMINS B

Members of the vitamin B complex, although not related chemically, are water soluble materials of importance in intermediary metabolism. A number of them are known to be cofactors essential for the functioning of enzyme systems, while the exact loci of action of other members of the group have still to be defined.

### Niacin

As is true of other B vitamins, niacin occurs in the diet and can also be synthesized by intestinal bacteria. The amide of niacin forms part of coenzymes I and II, and lack of the vitamin undoubtedly disrupts metabolic pathways which depend on these cofactors. In some species the amino acid tryptophan serves as the starting point for niacin synthesis. In man, lack of tryptophan exacerbates the signs and symptoms of niacin deficiency.

The syndrome resulting from niacin deficiency is termed pellagra; among its manifestations are alimentary disturbances, including diarrhea. In dogs on a niacin deficient diet, the gastrointestinal lining may be severely altered.[37] Pellagra perhaps also affects the myenteric plexus of the gut (Winckel, W. E. F.: Docum. neerl. et. indones. morbis trop. 3:100-104, 1951). Pellagra may be found secondary to alimentary diseases,[38,39] likely due to impaired absorption and elevated need.

**Riboflavin**

When phosphorylated, riboflavin functions as a coenzyme (flavin adenine mono- or dinucleotide), serving in the chain of intracellular electron transfer. When administered to rats, riboflavin concentrates in the mucous membrane of the intestine and is phosphorylated (possibly by one of the intestinal phosphatases[40]). Interestingly, the antibiotic chlortetracycline inhibits intestinal absorption of histidine,[41] and also complexes flavin adenine dinucleotide.[42] This suggest that one of the methods of action of chlortetracycline is inhibition of the coenzyme formed from riboflavin.

A number of years ago, it was reported that iodoacetic acid poisoning resulted in malabsorption[43] and that phosphorylated riboflavin reversed the effect; this has not been confirmed or extended. Riboflavin is also essential for maintenance of the enzyme xanthine oxidase in the gut.[44] Specific defects in the absorption of riboflavin are unknown, and in sprue, riboflavin, thiamine, and pyridoxine appear to be taken up normally.[45] Riboflavin deficiency does not appear to primarily affect the gut.

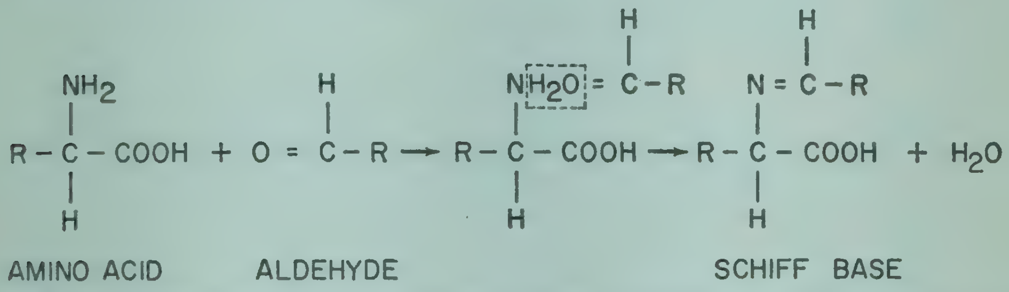
**Thiamine**

This vitamin can also be synthesized by intestinal bacteria and the synthesis is favored by the presence of sorbitol.[46] Thiamine is degraded by intestinal bacteria, usually in small amounts. Several bacterial species, however, have been reported to contain an active thiaminase which cleaves thiamine, with resulting thiamine deficiency[47] ("thiaminase disease").

Upon phosphorylation, thiamine is converted to a coenzyme essential in pyruvic acid oxidation. During marked thiamine deficiency (beriberi), an elevated blood level of pyruvic acid occurs—it can not be metabolized further in the absence of sufficient coenzyme. Patients with peptic ulcers may have high blood pyruvic acid levels,[48] probably reflecting the result of inadequate dietary vitamin intake and the severity of their disorder. Thiamine lack itself affects the stomach[49] and also increases choline esterase activity in the small intestine of pigeons.[50] In man, thiamine lack can be associated with diminished intestinal tone and motility.

**Pyridoxine**

In its phosphorylated form, this vitamin is a necessary cofactor for a number of decarboxylation and transamination reactions. There is evidence that amino acid absorption occurs through the formation of a Schiff's base,[51]



and that pyridoxal is the source of the aldehyde during the process. If this is substantiated, pyridoxal would emerge as one essential link in the amino acid absorptive mechanism. Pyridoxine deficiency has been noted to increase the uptake of iron from the gut of the rat,[52] perhaps in an attempt to overcome the anemia associated with lack of the vitamin. Human lack of pyridoxine, whether produced by a deficiency diet in infants, or use of a metabolic antagonist (desoxypridoxine), is accompanied by gastrointestinal symptoms.

**Pantothenic Acid**

This vitamin is a component of coenzyme A, an important factor in fatty acid oxidation and biological synthesis. Aside from occurring widely in food materials, pantothenic acid is synthesized by a number of bacteria. In rats, pantothenic acid deficiency is associated with duodenal ulcerations.[53] In man, d-pantothenyl alcohol has been used to decrease postoperative nausea and vomiting,[57] and in the treatment of paralytic ileus.[61]

**Biotin**

The role played by biotin in human nutrition is undetermined. Biotin, however, is unusual in the following respect. Avidin, a substance present in egg white (uncooked), combines with biotin,[54] forming a compound that is not absorbed from the alimentary lumen. Hence, even when sufficient biotin is ingested (it can also be produced by gut bacteria), a 'conditioned deficiency' results if excessive raw egg white is eaten. Human volunteers, ingesting large quantities of egg white (which also contains an antitrypsin), were observed to develop a number of symptoms.[55] In the rat, two biotin-like substances are found in the stool,[56] but their origin is uncertain. Biotin deficiency can be produced in the rat by a biotin-free diet only if coprophagy is prevented.[62] It is possible that the vitamin, produced by bacteria in the colon, is efficiently absorbed only from the upper reaches of the intestine. Such a phenomenon is likely operative for several other vitamins.

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# 16

# CATHARTICS AND TOXINS

Few medications are more abused than cathartics. Their use is often irrational and even dangerous. Various cathartics cause excessive potassium loss, interfere with carotene absorption, produce staining of the colon and kidney, and may result in absorption of foreign oils. Despite this, the number of medications employed as cathartics is legion, and each year new chemicals and combinations of old agents are described. Employment of cathartics is probably justified when cleansing of the lower bowel is necessary for examination purposes or to keep a bed-ridden patient from straining. Aside from their use in such situations and a few other specific instances, the potential harm they may cause outweighs any nebulous benefits from 'regularity.'

From the overwhelming and generally meaningless mass of available cathartics, we can somewhat arbitrarily divide these agents into four classes.

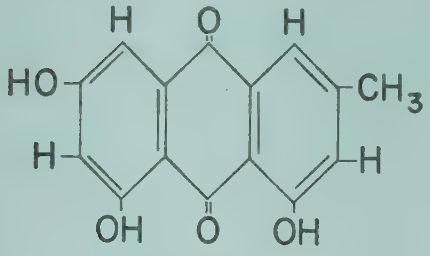
1. Irritants.
2. Bulk-increasing agents.
3. 'Lubricants' and surface active agents.
4. Stimulants of intestinal musculature.

**Irritants**

Common examples of irritant cathartics are the botanical drugs rhubarb, senna, aloe and cascara. These products contain free or glycoside-linked anthraquinone derivatives (principally emodin and chrysophanic acid)[1] (Figure 9).

When ingested as the glycoside, the compound is split in the alimentary lumen to the active free agent. Anthraquinone derivatives can be absorbed across the gut wall.

Continued use of anthraquinone derivatives as cathartics re-



EMODIN
(Trihydroxymethylanthraquinone)

Figure 9. Structure of emodin (trihydroxymethylanthraquinone).

sults in melanosis coli, an entity generally neglected in the literature. In melanosis coli, the large bowel shows brown granules on microscopic examination, and a macroscopic dark appearance.[2,47] Genesis of the condition is perhaps through oxidation of emodin and similar compounds, by intestinal bacteria, to pigments resembling melanin; these then become attached to proteins of the gut wall.[3] The degree of colonic staining is likely a function of the quantity and duration of cathartic use; the lesion may be reversible with time if laxative is withheld.[47] This type of bowel staining appears to be distinct from the pigmentation of intestinal smooth muscle fibers reported in some cases of malabsorption.[4]

Chronic use of cascara with production of loose stools results in potassium loss, ultimate deficiency,[19] and renal damage. The intestinal tract subjected to cathartics for many years may also show roentgenographic abnormalities,[20] perhaps due in part to potassium lack.

Another group of irritant botanical cathartics consists of resinous drugs which are slow in action and toxic in effect. Their use is accompanied by cramping and severe intestinal irritation. Such drugs (jalap, colocynth, podophyllum, gamboge, croton oil) have no place in present therapeutics. Still another cathartic of botanical origin is the active principle (dioxyphenylisatin) probably responsible for the peristaltic action of prune juice. It is commercially available,[45] as is diacetyldihydroxyphenylisatin, which is assumed to be a precursor of the prune-factor. The diacetyl compound is stated to be nonabsorbable and nontoxic.[5,6] The structural formula of the parent compound of this group (isatin) is given (Figure 10). Closely related chemical agents have a similar cathartic action.[40]

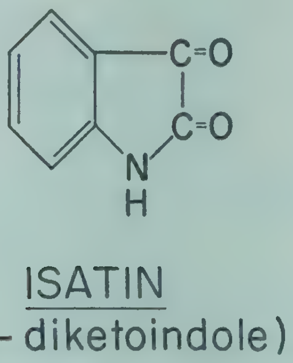


Figure 10. Structure of isatin (2,3-diketoindole).

Two widely employed irritant cathartics are phenolphthalein and castor oil. Phenolphthalein is nontoxic in most individuals, although some is absorbed, and is generally an effective cathartic. Castor oil, a triglyceride of an unsaturated hydroxy fatty acid ($C_{17}H_{32}OH \cdot COOH$, ricinoleic acid), most likely owes its action on the intestine to saponification and formation of sodium ricinoleate (an irritant to mucous membranes). Castor oil may produce a chemical gastroenteritis,[7] and in some individuals the degree of irritation is severe.

### Bulk-increasing Agents

Distention of the intestine is a normal stimulus for peristaltic activity. Agents which increase bulk thus enhance peristalsis in the healthy and nonobstructed gut. Two principal types of bulk-increasing agents are employed: hydrophilic colloids, and hydragogic salts.

Hydrophilic colloids (such as agar, plantago seed and its derivative metamucil) are not digested in the intestinal tract; they imbibe water, swell, and produce distention with little irritation. This type of agent is probably the least noxious of all the cathartics (but will cause difficulties when partial obstruction is present). It has been noted that psyllium seeds (Plantago psyllium) contain a principle which results in kidney pigmentation in rats,[8] but the significance of this finding is unknown. Methyl cellulose[9] and various polymers[10] have also been used to increase the intraintestinal bulk.

Hydragogic salts are soluble, yet poorly absorbed, such as magnesium sulfate. Probably by osmotic action, the salt solution calls forth water from the intestinal wall, thereby increasing the luminal bulk and initiating peristalsis. A number of hydragogues aside from magnesium sulfate are in use, including magnesium citrate, potassium sodium tartrate, sodium phosphate, and sodium sulfate.

### 'Lubricants' and Surface Active Agents

Oils which are not saponified within the lumen, such as mineral oil and many vegetable oils, exert a lubricating action on the fecal mass in the lower intestine. These compounds, however, exert no softening action on the stool and are not efficient stimulants of peristalsis. There are three disadvantages to their use.

1. Mineral oil interferes with absorption of carotene (the precursor of vitamin A [11]). During prolonged ingestion of mineral oil, the plasma level of carotene may severely decrease, with little accompanying change in the vitamin A level.[12]

2. Mineral oil may be fractionally absorbed across the intestinal mucosa and distributed throughout the body as a foreign material.[13] The degree of absorption depends upon the extent of subdivision of the oil droplets[14] and thus more is absorbed from an emulsion.

3. Mineral oil retards gastric emptying, and hence must be taken on an empty stomach.

A surface active agent employed in the treatment of constipation is dioctyl sodium sulfosuccinate (Figure 11). This material lowers the surface tension of the fecal mass within the lumen, allowing water and fatty materials to be better admixed with the bolus.[15] Dioctyl sodium sulfosuccinate thus permits softening of the fecal mass and facilitates its movement by normal peristalsis.[16] The compound itself is stated to be nonabsorbed and relatively nontoxic. The use of dioctyl sodium sulfosuccinate in conjuction with mineral oil is perhaps contraindicated, since the surface active agent may enhance emulsification of the oil and hence encourage oil absorption.[15]

Another group of surface active agents, the polyoxyethylene sorbitans ('Tweens') have enjoyed acceptance as emulsifiers for fats, but have not gained wide use as cathartics. These agents may

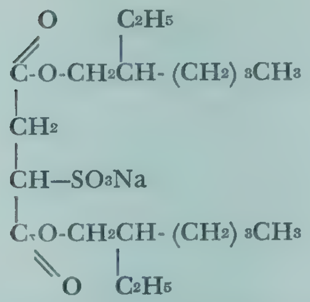


Figure 11. Structure of dioctyl sodium sulfosuccinate.

also alter absorption.[17] Attempts have also been made to soften the fecal mass by enemas containing surface active agents or enzymes.[18] Bile salts, because of their emulsifying action, probably also work as cathartics by enhancing the mixing of stool with intraluminal fluids.

### Stimulants of Intestinal Musculature

Posterior pituitary extract (see chapter on Hormones) directly stimulates smooth muscle of the alimentary tract. Its indiscriminate use, however, is dangerous as the hormone affects other organs as well as the gut and elevates the blood pressure. Ergot derivatives also stimulate intestinal muscles but are too toxic to employ for this purpose. Parasympathomimetic drugs (such as neostigmine) are stimulatory to muscles in the gut wall and have fewer side effects.

### Enemas

Introduction of fluid into the lower intestinal segment may initiate defecation by softening the fecal mass and distending the gut. Enemas consisting of water without added electrolytes are potentially dangerous, as they can leach potassium and electrolytes from the gut wall.[21] Absorption of part of the water introduced this way leads to a dilution of electrolytes within the blood stream.[22] Further, enemas may result in cardiac inhibition,[23] perhaps through reflexes initiated in the lower intestinal segment, and can rarely cause perforation of the bowel. It is thus apparent that cathartics and enemas should not be administered without due regard to their potential dangers.

## TOXINS

The great metabolic activity of the intestinal mucosa and its accessibility by both oral and parenteral routes renders this tissue particularly susceptible to noxious agents. For instance, anoxia has detrimental effects on mucosal activity (see section on Anoxia), and both folic acid antagonists[24] and nitrogen mustards[25] damage the intestine and its enzyme systems. Protein precipitants such as the heavy metals and phenols[26] severely damage cells facing the alimentary lumen. A number of other toxins are also of interest because of the insight they give, or experimental leads they provide, to intestinal function.

### 1. N-ethyl Maleimide

Two potent inhibitors of sulfhydryl groups are N-ethyl maleimide and iodoacetimide. When applied to the gastric mucosa, these reagents inhibit hydrochloric acid production.[27] This is not surprising, since secretion of acid requires work and undoubtedly an enzyme network as well (which may contain one or more -SH groups). What is surprising, however, is that 'mucous' production is not inhibited by use of these reagents. Even though mucous synthesis undoubtedly involves expenditure of energy and the mediation of enzymes, the system is not particularly sensitive to N-ethyl maleimide. This further illustrates that the gastrointestinal canal performs multiple tasks and that inhibition of one function does not necessarily imply cessation of all activities.

### 2. Carbonic Anhydrase Inhibitors

Not only are alimentary enzymes involved in the production of digestive ferments, but certain systems doubtlessly control, at least partially, the composition of gastrointestinal secretions. Carbonic anhydrase is the enzyme catalyzing the reaction:

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-.$$

This enzyme has been implicated in both pancreatic production of bicarbonate[28] and gastric manufacture of hydrochloric acid;[29] both activities are interfered with by inhibitors of carbonic anhydrase. These observations point the way to exploring the uncharted region of the control of the composition of alimentary secretions.

### 3. Detergents

As discussed in the chapter on Intestinal Bacteria, the gut mucosa is 'impervious' to a number of toxic agents—including histamine. The mucosa contains histaminase which inactivates histamine. The application of detergents to the intestinal mucosa of animals so damages the cells, or certain components of the cells, that histamine can no longer be degraded. Histamine injected into the lumen then enters the body with the production of lethal symptoms.[30] The role of the mucosa in forming a first line of defense against the entry of noxious materials is little understood.

### 4. Colchicine

This interesting pharmacologic agent has antimitotic properties, and has been used to stop the division of mucosal cells in animals.[31] When administered to man, as during the treatment of gout, gastrointestinal reactions may be severe. Because of these undesirable effects, colchicine derivatives with less alimentary toxicity have been employed.[32] In very low doses, colchicine affects the mitotic index of the crypts of Lieberkuhn in the guinea pig intestine[33] (these cells have a high rate of replacement and also appear to be the most sensitive to the effects of irradiation). Colchicine may also affect the level of intestinal alkaline phosphatase.[34]

### 5. Parasitic Toxins

The bacteria Salmonella typhi has been shown to produce an endotoxin, which upon intracardiac injection causes edema and congestion of the intestine, as well as engorgement of mesenteric vessels.[35] Microscopic mucosal hemorrhages occur, and stools give a positive reaction for occult blood. Within 30 seconds of the injection of endotoxin, there is a 75 per cent decrease in oxygen consumption of small intestine mucosal homogenates. The worm Ascaris,[36] the staphylococcus[37] and the dysentery organism[38] all produce toxins which adversely affect the intestine. Study of the mechanisms involved is in an early state of development.

### 6. Gossypol

Cottonseed contains a complex material termed gossypol.[39,48] There are reports that administration of gossypol to animals is accompanied by intestinal hemorrhages. Rigdon and coworkers[40] found that in young chickens, gossypol administration caused a hemolytic anemia as well as the deposition of a ceroid-like pigment in the duodenal villi and elsewhere. Such studies, using the intestine as an experimental organ may shed light on the origin of ceroid storage disease.[49] Ceroid is also deposited in the gut during tocopherol lack.[50]

### 7. Salicylates

A number of reactions are shown by the gastrointestinal tract upon administration of salicylates. Peptic ulcers are aggravated,[41] mucosal hemorrhages occur,[42] and the ability of the mucosa to transport materials is depressed.[43] Salicylates may uncouple phosphorylation, but how much this effect contributes to the actions of salicylates on the gut is unknown.[44] It is quite startling to find that a compound so frequently administered has such drastic effects on the intestine.

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# III

# DISORDERS OF INTESTINAL FUNCTION

# 17

# SOME EFFECTS OF DIET AND PSYCHE

A DISCUSSION OF THE effects of emotional factors on the alimentary canal is all too likely to be brushed aside as being 'unscientific' or 'naive.' What is meant by these terms is that the specific pathway between the emotion and the gastrointestinal reaction can not always be defined in exact biochemical and anatomical terms. In many cases, this reflects our ignorance, rather than lack of a cause-effect relationship. At the other extreme, blind acceptance of emotional factors as the cause of all alimentary disorders of otherwise unknown etiology serves no purpose other than to discredit its proponents. Several basic factors have been recognized as being important in the psyche-alimentary relationship.

1. Through the vagus nerve and fibers from the spinal cord, the brain has an anatomic pathway to the alimentary tract. Both afferent and efferent impluses pass between brain and gut.

2. The brain contains 'areas' which influence gastrointestinal function. Brain damage can be accompanied by the development of alimentary abnormalities.

3. Studies of the human gastrointestinal apparatus by means of fistulae[1,2] have shown variations in motility, blood flow, and secretions in response to changing emotions. It must be pointed out, however, that although such changes can be marked, they have not been observed to progress to frank disease.

4. A few alimentary disorders have been closely tied to emotional factors. While perhaps not the only mechanism responsible for the changes, the psyche has been implicated in the genesis of: nervous vomiting, anorexia nervosa, nervous diarrhea, constipation, irritable colon,[3] and perhaps recurrent abdominal pains.[10]

5. In alimentary disorders clearly not emotional in origin, the

patient is often greatly helped by psychotherapy. The conclusion that we are unable to isolate a human being from his emotional environment is obvious. Studies reporting improvement induced by psychotherapy, in persons suffering from alimentary disorders of obscure etiology, are thus generally quite true. However, sweeping conclusions that the psyche engendered the disorder should be carefully avoided. A sequel of this is that intelligent guidance of the patient, and the searching out of his problems, may contribute to overall handling of the case. The results of therapy in one series handled in this manner by physicians was as good as in cases specially treated by psychiatrists.[4] Introductions to the vast literature on psychosomatic aspects of alimentary disorders are provided by Lief[5] and Dunbar.[6]

Little understood is the role played by the intestine in the regulation of eating habits. Hill and coworkers[7] reported that introduction of hypertonic glucose into the intestine of a dog resulted in reduction of food intake; crude fibers were somewhat effective in this respect while tyrosine was ineffective. Perhaps distention[8] or muscular activity resulted in a dulling of the desire to eat. Interestingly, food taken orally appears to be a better reward for animals than similar materials introduced directly into the stomach.[9]

Psychic factors and altered eating habits have been mentioned as being of possible significance in the development of food intolerance and food allergy.[11] Allergic responses on the part of the alimentary canal have been mentioned in connection with malabsorption[12] and diarrhea;[13] much additional evidence is necessary, however, before any relationship can be assumed to exist. Eosinophils occur in the stool and have been identified with 'allergy.'[14] However, eosinophils normally occur in the gut wall,[15] although the stimulus for their entrance into the lumen is uncertain. Charcot-Leyden crystals,[16] formed from eosinophils, can be observed in the stool during parasitic infections and other conditions, but their significance is also obscure.

Not only eating habits, but the type and quantity of food taken affect the gut. Even the weight of the gut varies with the diet ingested.[17,18] As discussed in other chapters, the composition of digestive secretions elaborated depends in part upon the diet. Fasting decreases 'energy rich' phosphate compounds in the gut,[19] and depresses the production of pancreatic enzymes.[20] Adequate feeding reverses these changes. Simple deficiency of amino acids can also affect the alimentary apparatus.[21] Most cases of malnutrition, however, are the composite of multiple deficiencies.[22]

A disease of malnutrition which has received much attention since 1950 is termed kwashiorkor. The condition is observed, most commonly, in young native children of tropical regions, who are raised on a diet poor in all respects, especially protein. There is growth failure, hypoproteinemia and edema, skin changes, infiltration of the liver by fat, and impaired gastrointestinal function. Intraluminal enzymes are depressed, and their counterparts in the blood stream are also low. Enzyme secreting cells (particularly of the pancreas) are impaired; amino acid absorption can be almost normal in affected individuals, while vitamin A absorption is often defective.[25] Pathogenesis of kwashiorkor is uncertain, and probably involves multiple deficiencies along with inadequate protein intake. Superimposed diseases, such as hookworm infestation, contribute to the general debilitation. Good introductions to the subject are provided in two monographs.[23,24] Following refeeding of protein-rich foods, the symptom complex regresses and intraluminal enzymes return toward the normal level. Many children relapse when removed from dietary control and returned to their deficient intake.

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# 18

# ULCERATIVE COLITIS

As is true of most diseases of obscure etiology and uncertain treatment, an extensive literature has appeared concerning ulcerative colitis. Summary volumes on the subject have been contributed by Bargen[1] and Bacon.[2] Since there are no pathognomonic signs, diagnosis is made on the basis of clinical symptomatology (bloody diarrhea, cramping, malaise, anorexia, fever and weight loss), proctoscopic findings and x-ray data (decreased distensibility of the colon, narrowing, ulceration[54] and the formation of pseudopolyps between sloughed areas). There may be extracolonic manifestations of ulcerative colitis.[49] This indicates either that the illness is part of a more fundamental general disturbance, or that the colonic disorder engenders widespread alterations.

A major difficulty in studying ulcerative colitis as an entity is the fact that 'typical' findings are encountered in other pathologic processes. Ulcerative colitis may be simulated by "specific" disorders[3] or may occur during the course of another disease.[4,44] Further, fairly similar lesions are produced by a number of experimental techniques.[45,46] Since ulcerative colitis can be acute or chronic, and undergoes spontaneous variations, the effect of medications is difficult to judge unless a large series of patients is studied. The disease usually occurs in children and the 20 to 40 year age group. Its onset in young individuals, and the prolonged course, make long term follow up of ulcerative colitis a possibility. This ability to follow the disease over many years, and the reported finding of an increased incidence of carcinoma of the colon in association with ulcerative colitis,[5] makes the illness of particular interest to oncologists.

The etiology of ulcerative colitis remains undetermined. Some of the most recently advanced theories[6] are as follows.

### 1. Infection

Such a causative agent is suggested by the febrile nature of the illness, frequent abscess formation, colonic inflammation, and occasional response to sulfonamides. There has been speculation that infectious agents are only indirectly involved; their toxins or enzymes could damage the mucosa and enhance the ability of other agents to irritate the colon. The possible role of viral agents in ulcerative colitis is unknown.

### 2. Nervous System Effects

There is recognition that emotional factors produce alterations in the motility, blood flow, and mucous production of the colon.[7] Whether these effects are of importance in the genesis of ulcerative colitis is uncertain; the role of psychic factors in the development of the disorder is far from being agreed upon.[8,48] Alimentary ulcerations have been noted in monkeys with electrodes implanted intracerebrally (permitting self-stimulation [9]). The observation has been made that the number of automatic nerve ganglia in the bowel wall is apparently increased in ulcerative colitis.[6] This, in turn, suggests some aberrant action of the nervous system in producing ulcerative colitis. Prolonged administration of parasympathomimetic drugs to animals results in a picture resembling ulcerative colitis,[10] and suggests a possible role for the parasympathetic nervous system in the genesis of the naturally occurring disorder.

### 3. "Hyperreactivity"

Serum mucoids are elevated during the course of ulcerative colitis;[11] the same sort of elevation is seen in the "collagen" diseases. However, alterations in blood proteins and enzymes during the disease[47] may reflect tissue injury or the effects of inanition, rather than changes directly referable to the basic disorder. Recently the allergic aspects of the disease [57,58,59] as well as an abnormal response to injury have been stressed.[56]

Since there is sparse information as to the causation of ulcerative colitis, no well defined therapeutic approach has evolved. Symptomatically, the objectives of treatment have been stated to be as follows.

1. Decreasing activity of the colon,
2. Maintaining nutrition,
3. Eliminating infection.

Activity of the colon is reduced by ingestion of a diet which leaves little residue for excretion[8] (such diets can be selected by the use of tables listing the average crude fiber per gram of food [12]). Rest, perhaps sedation and psychotherapy may also contribute to decreasing colonic activity. The use of tranquilizers is uncertain. Rauwolfia serpentia and derivatives, which liberate serotonin (a smooth muscle stimulant), are probably contraindicated as sedatives.

The maintenance of nutrition may be a difficult problem during the course of ulcerative colitis. As will be discussed below, there is protein and electrolyte loss from the gut; to further compound the problem, absorption of a number of nutrients may be decreased during the active stage of the disease. Supplements of nutrients are in order, and blood transfusions may be necessary to correct anemia and hypoproteinemia during exacerbations. Sulfonamides and antibiotics have been used in the treatment of ulcerative colitis. While such medications likely exert no fundamental action on the basic disease process, they can prove beneficial in many cases by controlling attendant infections.

ACTH and cortisone were tried in the therapy of ulcerative colitis in 1950. These medications are not curative but can result in clinical improvement by controlling inflammation and creating euphoria. Complications of the steroids when administered in the course of ulcerative colitis are many. These adverse reactions, as well as the indications for steroids in the treatment of ulcerative colitis are discussed by Truelove[53] and Zetzel.[13] An interesting development has been the slow rectal infusion of hydrocortisone during active stages of the disease.[15,16] Since cortisol-$C^{14}$ administered in this manner is not significantly absorbed,[17] it is likely that clinical benefits from intrarectal steroids result from local action of the medications. This route of administration has the advantage of resulting in almost no side effects, since the drug does not enter the general circulation. (Other work however indicates that there may be some absorption, from the rectum, of cortisol [55]).

Surgery is employed in ulcerative colitis when there is profuse bleeding, obstruction, perforation, or a rapid downhill course. Other indications for surgical intervention, the types of procedures used, and prognosis, are discussed by Kirsner and coworkers.[8] Since it has been shown that excessive quantities of parasympathetic medications produce lesions similar to those of ulcerative colitis, surgical vagotomy (to reduce parasympathetic stimuli to the upper portion of the large colon) has been tried.[14] Although pregnancy may exacerbate ulcerative colitis, patients can often conceive and carry through a pregnancy after surgical intervention.[39]

A number of biochemical alterations accompanying ulcerative colitis have attracted attention.

### Lysozyme

Fleming[18] noted an enzyme (termed lysozyme) in nasal secretions, tears, saliva, and gastric juice. The enzyme has mucolytic activity and is assayed by following the reduction in viscosity of a polysaccharide prepared from Micrococcus lysodeikticus.[19,32] Histamine,[20] parasympathomimetic drugs, and sympathectomy do not increase lysozyme secretion.[21]

Lysozyme can be demonstrated in the alimentary canal and feces. Fecal excretion of the enzyme is greatly increased in ulcerative colitis.[23] The level of the enzyme excreted roughly correlates with the activity of the disease; as the acute phase subsides, the lysozyme titer approaches normal. It has been shown that abrupt increases in the lysozyme titer of patients with ulcerative colitis may accompany mental agitation.[24,25]

As convincing as this evidence sounds, there is good reason to believe that lysozyme is not of importance in the etiology of ulcerative colitis. Following prolonged exposure to lysozyme, colonic mucosa shows no alterations.[26,27] It is likely that lysozyme has no substrate in the colon.[28] Even when injected into mesenteric vessels, lysozyme produces no changes in the colon.[33] However, while lysozyme does not digest intraluminal mucous, its effect on intracellular mucous is unknown.[29] ACTH and cortisone, administered during an exacerbation of ulcerative colitis, produce a decrease in fecal lysozyme which parallels clinical improvement. However, inhibition of fecal lysozyme by oral administration of a detergent does not result in clinical improvement. Such observations suggest that fecal lysozyme is not the basic defect in ulcerative colitis, but rather reflects the response of the colon to injury.[29] Support for such a concept has also been forthcoming from the demonstration of lysozyme in granulation tissue, pus[30] and granulocytes.[31] Steroid therapy likely reduces lysozyme excretion by diminishing granulation tissue and the number of blood cells in the stool. However, fecal lysozyme excretion remains one of the few available parameters for evaluating colonic activity during the course of illness, and its level approximately correlates with the state of activity of ulcerative colitis.[34]

**Protein Loss**

Leakage of blood from the ulcerated colon, and the sloughing of tissue, depletes the body of protein. In addition, protein-rich digestive fluids may be passed during episodes of diarrhea, placing the body nitrogen economy in further jeopardy. For example, each pint of blood lost depletes not only hemoglobin, but also plasma proteins; each gram of tissue sloughed carries away about 0.2 grams of protein, and each 100 ml of digestive fluid passed contains about 0.4 grams of protein (see chapter on Protein Loss).

Patients with ulcerative colitis may possess a total exchangeable albumin-$I^{131}$ body pool that is much smaller than the normal value of approximately 360 grams. Following administration of radioactive albumin to such patients, there occurs a 10 to 50 per cent fecal loss of the radioactive tag.[35] The small albumin pool may thus be due to prolonged fecal loss and inadequate replacement. Immunologic techniques demonstrate the presence of typical blood albumin and globulin in the gut lumen of patients suffering from active ulcerative colitis,[35] showing that there is a true protein 'leak' across the mucosa. When this loss is added on to poor dietary intake and possible reduced absorption, it can be understood how a pan-tissue protein deficit develops.

### Potassium and Electrolyte Loss

Not only are alimentary secretions lost in ulcerative colitis, but tissue and blood also escape in the feces. The loss of electrolytes in general, and potassium in particular, during the acute phase of the disease, is a serious complication whose importance can not be overemphasized. I am well convinced that many of the manifestations of malaise and debility shown by such patients are referrable to depletion of potassium (see also chapter on Potassium Metabolism). The case of a middle age woman is particularly called to mind. She died a few hours after entering the hospital, despite therapy. Her blood level of potassium was 2 milliequivalents per liter (compared with a normal value of 5) and intracellular potassium, determined by analysis of tissues by biopsy, was also reduced. The management of electrolyte disturbances in these patients is particularly complicated if adrenal steroids are being administered.

### Absorption Studies

There has been a tendency to assign many of the nutritional difficulties of patients with ulcerative colitis to 'malabsorption.' The evidence for this, however, is not clear. Certainly absorption of nutrients is complete before the colon is reached, and it is difficult to envision how colonic disease would impair absorption. A number of suggestions present themselves for consideration.

1. A number of patients with ulcerative colitis have involvement of the ileum. Vitamin $B_{12}$ absorption occurs in the ileum[51] and some lipids may also be absorbed in this area;[52] hence ileal disease[36] may be the true source of the absorptive error. There also remains the possibility that the grossly disordered colon is but one manifestation of a disease which affects all levels of the gut.

2. Perhaps reflexes from the damaged colon diminish the activity of other areas (it is known, for instance, that such a relationship likely holds between the stomach and duodenum [40]). Colonic disease might result in hypermotility or other aberrations sufficient to diminish absorption.

3. The intestinal tract may suffer from a chronic state of malnutrition during the disease, with resultant dysfunction and malabsorption.[42]

The uptake of vitamin A has been reported to be diminished in ulcerative colitis.[37] However, as pointed out in the chapter on Vitamins, this is a poor test substance to use, since it can be absorbed into the mucosa but not discharged into the blood stream until a short time later.[41] Following ingestion of methionine, a low blood level occurs in patients with ulcerative colitis—but this has been ascribed to rapid use of the compound. However, another study reported increased fecal excretion of labeled methionine after ingestion by patients with ulcerative colitis,[38] and the nature of the defect remains unsettled. Radioactive fat excretion has been reported diminished in these patients,[38] while galactose absorption was increased.[37]

### Hepatic Function

During ulcerative colitis, the liver is embarrassed by protein loss and electrolyte wasting. When added to poor oral intake in debilited individuals, frank liver dysfunction can occur. In addition, bacteria likely enter the portal circuit through the damaged mucosa.[43,50] All of these factors, plus general toxicity, play a role in contributing to hepatic disease during the course of ulcerative colitis.

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# 19

# REGIONAL ENTERITIS AND PSEUDOMEMBRANOUS ENTEROCOLITIS

In 1932, Crohn, Ginzburg and Oppenheimer[1] described 14 cases of intestinal inflammation involving the terminal ileum. This 'regional ileitis' was noted to be an entity distinct from a large number of "benign granulomas" of the intestine. Description of the disorder as a regional ileitis was later shown to be too limited, as similar lesions were found in other areas of the alimentary canal (from stomach to rectum). Hence, 'regional enteritis' has been proposed as a more suitable descriptive term. Crohn has published a summary of his experience with the illness,[2] and a number of reviews have appeared.[3,14,15,16,17,19]

Clinical findings in the disease are understandable in terms of the pathologic processes involved. In regional enteritis, inflammation of the gut produces ulcerations and sloughing. Responding to this insult, a connective tissue reaction occurs, which in turn leads to stenosis of the lumen. Inflammed intestinal loops become matted together, with a resultant abdominal mass. Affected loops can break down, forming fistulae between one section of the gut and another, or between the intestine and the abdominal wall. The finding of fistulae between intestinal segments should focus attention on a neoplastic process or regional enteritis, since 'benign' causes are uncommon.[5]

Regional enteritis has no single pathognomonic sign. Diagnosis early in the illness is difficult; fever, diarrhea, weight loss, blood and mucous in the stool, and anemia also occur in other conditions. Physical findings can include a mass in the right iliac region due to hyperplastic and inflamed ileal loops, and the above mentioned evidences of fistula formation. X-ray studies are sometimes helpful in pointing out the extent of the process, especially when the disease 'skips' areas (that is, when affected regions are interspersed between apparently healthy segments). Roentgenographic studies of the small bowel can provide the key to correctly recognizing the cause of unexplained symptoms. The most characteristic X-ray finding, after regional enteritis has been well established is a thin, smooth, narrow ileal lumen (termed the 'string sign' by Kantor [4]). Such a finding goes a long way toward making the diagnosis; other conditions causing similar changes in the x-ray picture of the ileum (tuberculosis, sarcoma) are uncommon.

The etiology of regional enteritis is unknown and multiple factors have been discussed as potential causative agents. Giant cells, and other evidence of a chronic granulomatous reaction in regional enteritis, have suggested a relationship to sarcoidosis or tuberculosis. Some investigators are of the opinion that the giant cells found in the thickened submucosa and local lymph nodes, as well as the proliferated endothelium and obstructed lymph pathways, are rather specific for regional enteritis (see review by Zetzel [3]). Apparent similarity of the lymphatic obstruction to that produced by infectious agents (primarily filaria) or foreign bodies (silica, talc), has led to attempts to produce regional enteritis in experimental animals. Somewhat similar naturally occurring diseases have been described in pigs[6] and cocker spaniels,[7] but an etiologic agent could not be isolated. Recrudescence of regional enteritis after bacterial infection has brought forth proposals of specific infectious agents, or the reaction to infection, as an initiating process in the disease. Possible relationship of the lesions to mucosal anoxia is suggested by a case report of regional enteritis simulated by incomplete intestinal infarction.[11] Emotional factors have also been discussed as being of importance in the genesis of regional enteritis, but additional data is needed. A most interesting finding has been the occurrence of aberrantly placed Brunner-type glands in resected areas of the intestine in certain patients with regional enteritis.[9] It is known that normally situated Brunner's glands can undergo cystic dilatation during chronic irritation,[8] but abnormally placed glands have not been described in conditions other than regional enteritis. The recurrence rate of the disease was highest in patients who had the misplaced Brunner's glands.[10]

The ileum appears to be vulnerable to attack by both regional enteritis and by an extension of the lesions of ulcerative colitis. The differential diagnosis can thus be difficult; Bargen has discussed the essential clinical points.[12] Jackson noted ulcerative colitis to occur in 11 per cent of individuals afflicted with regional enteritis,[17] suggesting a close relationship between the disorders.

Therapy, since the etiologic agent is unknown, is symptomatic and supportive. Because of intestinal inflammation and general toxicity, adrenal steroids have been used; however, the response to these medications is often not pronounced.[16] Chemotherapy is likewise often ineffective. Resection of diseased bowel, and side-tracking operations have been employed during the course of regional enteritis. Patients who have more than 10 feet of intestine removed do not fare well clinically.[16] Perhaps this is because massive resection is only carried out in individuals with extensive disease. A contributory factor, however, may be the absorptive errors which follow such a procedure. It has been demonstrated that, in man, the ileum is a major site of vitamin $B_{12}$ absorption.[18] Hence, ileal disease, or ileal resection, can likely result in deficient vitamin $B_{12}$ uptake. Indeed, 60 per cent of the patients with regional enteritis, in one series, had low blood levels of vitamin $B_{12}$.[13] The extent of the interference with absorption of other nutrients and electrolytes in this disease has been less well studied. Water and electrolyte wasting is not uncommon in regional enteritis, due to the loose bowel movements and loss of blood. As pointed out in the chapter on Protein Loss, this carrying away of blood (and fragments of tissue) depletes the body of protein, and disturbs an already marginal dietary balance.

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## PSEUDOMEMBRANOUS ENTEROCOLITIS

In the last decade, attention has been called to the occurrence of a dramatic disorder termed pseudomembranous enterocolitis. Robbins[1] has described a pseudomembranous inflammation as one ". . . characterized by the formation of a false membrane made up of precipitated fibrin, necrotic epithelium and inflammatory white cells." Such changes can occur in the throat during diphtheria; by analogy, cases of pseudomembranous enterocolitis were often referred to as 'diphtheritic colitis.' Most frequently involved is the large intestine, less commonly, the small intestine and stomach are affected.

Lesions typical of pseudomembranous enterocolitis were noted for many years before the advent of antibiotics. Chemical toxins and massive infections were the commonest probable causes. In the period since 1950, there has been an apparent increase in the incidence of the disease; this has been related to the use of antibiotics and the emergence of staphylococci resistant to these drugs. Although some series of cases do not support the contention that the disease has increased in incidence,[2] many more cases have been reported since 1950 than before. The overgrowth of staphylococci or proteus, when the normal bacterial flora is disturbed by antibiotics, may produce an enterotoxin which damages the intestinal mucosa. The opinion has also been expressed[3] that the basic pathologic process is an inadequate response to stress, with tissue breakdown and subsequent bacterial overgrowth. Experimentally, the lesion can be produced in chinchillas infected with staphylococci which produce an enterotoxin.[10]

A typical clinical picture consists of a patient on antibiotics (commonly postoperatively) who, after several days of apparently doing well, develops a fever, rapid pulse, abdominal distress, and mild diarrhea. At this point in the illness, diagnosis is uncertain unless developing pseudomembranous enterocolitis is considered. Stool cultures reveal a change in the bacterial content, with staphylococci now predominating. If untreated, the patient may deteriorate rapidly. Fulminant diarrhea with water and electrolyte loss develops. This is followed by peripheral vascular collapse, shock, and death. A particularly dramatic occurrence is the passage of a 'cast' of the intestine, made of necrotic cells, fibrin and bacteria. The major point in therapy is prevention. This requires careful consideration when antibiotics are used; they are administered only when needed, and only for periods necessary to combat the disorder being faced. A high index of suspicion is necessary to detect changes in the stool. Direct smear and Gram-staining of the feces is invaluable in showing the presence of staphylococci. Treatment of pseudomembranous enterocolitis is heroic.[4,5,6,7] Blood pressure is maintained by use of a pressor substance such as norepinephrine, and oxygen is administered if there is collapse, or if cyanosis is present. Fluid and electrolytes lost in the stool, and from other sources, are replaced. Broad spectrum antibiotics are discontinued, and are replaced by chloramphenicol or erythromycin[7] or other agents[9] potentially capable of stopping the growth of the staphylococci. There is also a report of administering normal intestinal bacteria by enema to restore the flora of the gut.[8]

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## 20

# ISOTONICITY AND THE DUMPING SYNDROME

DIGESTION AND absorption are influenced by the osmolarity of ingested metabolites, and changes in the osmolar concentration of substances taken by mouth have profound implications in the body economy. This is because homeostatic mechanisms are mandatorily called upon to maintain isotonicity in two phases (extracellular fluid and intestinal mucosal cells) at the expense of gut lumen fluids and the urine.

Cells of the gastrointestinal tract have an 'osmotic tonicity'[1] but there is no clear evidence that this changes when solutions of varying osmolar concentrations are in the lumen. However, the total composition of the cell probably depends, within limits, upon the dietary history of the individual, and it has been demonstrated in animals that even the weight of the gut varies with diet.[2,24]

There is perhaps a minimal ratio of water/food in the gastrointestinal lumen for proper digestion of food. When only small quantities of oral fluid are available, water must be mobilized from the milieu interieur to the gut lumen. 'Dry' diets decrease the appetite of rats, and effect a reduction of food intake.[3] The specific mechanism is not known, but may be related to the processes working to conserve water.

During 'dry' diet consumption, the body is faced with a difficult situation in partitioning water. Without fluid in the gut lumen, digestion may not proceed normally, but if fluid is drained from internal spaces, there is the danger of dehydration. The situation is actually not as drastic as this, since fluid and electrolytes in the intestinal lumen are in rapid interchange with their counterparts within the body. Indeed, electrolytes of the lumen are included in the pool of 'exchangeable' anions and cations, as measured by radioisotope dilution studies. The author's calculations (see Chapter on Potassium Metabolism) indicate that a volume of fluid as large as 1.9 to 2.8 liters may be present within the lumen of the normal adult gastrointestinal tract. It is obvious that loss of much of this water would impair homeostasis. Such considerations concern the responsibility of the body in supplying water for digestion and absorption. What of the other viewpoint, the effect of ingested water on these mechanisms? While the internal surface of mucosal cells is in contact with a buffered fluid, their external side *may* by in contact with a liquid that is not isotonic and is rapidly changing in composition. The word *may* in the preceding sentence is of some importance, for in the normal digestive tract, there are mechanisms that tend to bring ingested materials within a certain range of osmolarity (and it has even been claimed that the feces are isotonic [25]).

By the use of multiple-lumen tubes, which permit isolation of intestinal segments and the introduction or removal of fluids, it has been found that the ability to dilute hypertonic solutions within the lumen is possessed by the stomach, but to even a greater extent by the duodenum (which can bring concentrated glucose solutions toward isotonicity). However, experiments have shown that isotonic solutions placed in the ileum are altered[7] (toward hypotonicity), and seriously question theories which state that the intestinal contents are always brought to isotonicity. The intestine may perhaps also have the ability to alter hypotonic solutions in the lumen, since electrolytes can be shown to move from blood stream to lumen[6] but a net flow is difficult to prove. The mechanism responsible for the maintenance of (and deviations from) osmolarity is uncertain. There has been much speculation as to chemoreceptors and osmoreceptors in the gut wall, but little confirmatory evidence. The absorption of water from the lumen is at least partially an energy requiring process which can be supported by glucose.[8] Water uptake from the alimentary lumen is also dependent, in part, on hormonal factors. Adrenalectomy is accompanied by a decrease in the ability of intestinal mucosal cells to take up water and alter the osmotic pressure of isotonic solutions placed in the lumen.[9,10] These errors are corrected by steroid administration. Factors operative in the transfer of water in the opposite direction, from cells to lumen, are also uncertain. Information in these preceding paragraphs, incomplete though it may be, is necessary for an understanding of a clinical entity (or entities) called the 'Dumping Syndrome.'

Subtotal, and occasionally even total, gastrectomy has been utilized in the treatment of diseases of the stomach. The lowered surgical mortality in such procedures has resulted in the creation of a large population of postgastrectomy patients. Sequelae of this therapy are numerous (megaloblastic anemia, iron deficiency, a defect of fat absorption), and include a particularly disturbing complex called the 'Dumping Syndrome.'

The first phase of the symptom complex becomes evident within fifteen minutes of a meal. This 'early' dumping syndrome is characterized by fullness in the epigastrium, and any or all of the following: sweating, weakness, nausea, eructations, palpitations, pallor, hypotension and even collapse. Such an episode may last from ten to ninety minutes, can vary in different patients from mild to disabling and is likely to be brought on by a meal high in carbohydrates. It is often relieved by lying down, and vomiting may terminate the attack. A 'late' dumping syndrome occurs, usually following a meal by one or two hours, and may be merged into the early symptoms.

The Dumping Syndrome derives its name from the fact that a barium meal is quickly 'dumped' from the post-gastrectomy stomach remnant into the small bowel. The stomach usually provides an elastic reservoir for holding food, churning it into chyme, and periodically discharging it into the duodenum. Surgical removal of a major portion of the stomach allows ingested material to pass directly into the small gut. It is also known that the normal duodenum can initiate a reflex which delays gastric emptying, whereas draining the duodenum increases gastric motility and hastens gastric evacuation.[11]

Some investigators have doubted an organic explanation of the dumping syndrome.[12] Even if psychic factors are of major importance,[26] the syndrome is a rather frequent phenomenon, associated in some manner with rapid gastric emptying. As will be pointed out, there are marked differences of opinion concerning the etiology, and hence treatment, of this condition.

From the multiple theories advanced at one time or another, two distinct lines of thought have evolved as to the cause of the syndrome (see review by Webber *et al.*[13]).

1. The first concept states that the condition is related to the anatomical arrangement of the digestive canal after gastrectomy. Symptoms are thought to arise:

(a) From distention of the efferent jejunal loop, due to rapid gastric pouch emptying.

(b) From stretching of the jejunum due to the inpouring of biliary and pancreatic fluids.

(c) And from traction of the distended loop on the stomach.

Correction of these abnormalities has been attempted by converting Billroth II gastrectomies to Billroth I types with narrow stomata. At reoperation, support may be provided for the jejunum, and the size of the gastric pouch can occasionally be increased through the use of segments of colon or jejunum. The occurrence of the dumping syndrome after jejunal feeding of patients with intact stomachs, and the failure of balloons distended within the jejunum to exactly mimic the syndrome (it may result in epigastric pain), speak against mechanical factors as the sole cause of the condition.

2. The second line of reasoning has tried to link the dumping syndrome with one or more of the biochemical changes occurring after gastrectomy (such as sideropenia,[14] lack of intrinsic factor,[15] altered fat uptake,[16,17] and adrenal stimulation[18]).

Many of the signs and symptoms of the syndrome resemble a discharge of the sympathetic nervous system ("autonomic storm"), but neither ganglionic blocking agents nor splanchnicectomy give complete relief. Further confusing the issue is the fact that radiologic evidence of dumping occurs in many patients without symptoms.

Three additional abnormalities have been associated with the dumping syndrome, and have therefore been studied as possible etiologic agents. These are:

1. Hypoglycemia.[19]
2. A fall in the serum potassium ion concentration.[20]
3. A decrease in the total circulating blood volume.[21]

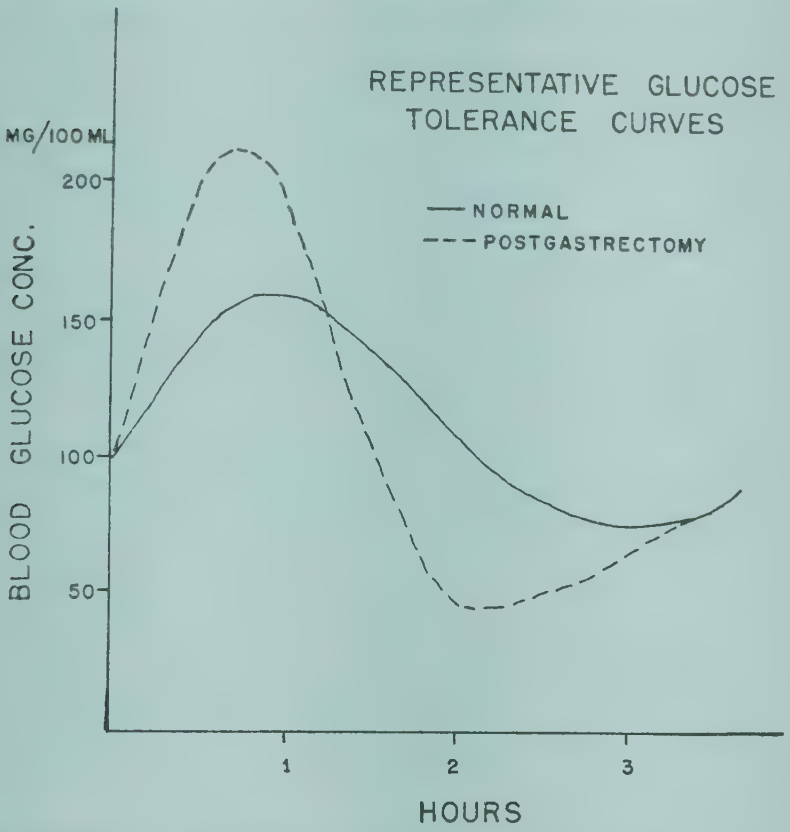

Figure 12. Oral glucose tolerance curves in a normal adult and a postgastrectomy patient. (Courtesy of the Navy Medical Department.)

The glucose tolerance test after gastrectomy may be distinctly abnormal (Figure 12) with hyperglycemia during the first hour (sometimes sufficient to result in glycosuria). The hypoglycemic phase which follows usually does not begin until after the first hour, and while it may account for the 'late' symptoms in certain patients, it is not an explanation for symptoms appearing within a few minutes of a meal. Electroencephalographic changes can occur during the dumping syndrome,[22] and while the exact mechanism is unknown, it may perhaps be related to hypoglycemia or a defect of potassium metabolism (q.v.).

A fall in plasma potassium during the early stages of 'dumping' has been noted[20] sometimes coupled with electocardiographic changes (questionably of hypokalemia). However, these changes do not occur in all patients with dumping symptoms. In addition, the drop in potassium is usually slight (0.5mEq/Liter), and may occur with or without symptoms. It has been stated[13] that the fall in potassium is not related to the development of the syndrome, but is rather the response to the introduction of hypertonic glucose into the jejunum. As sugar is absorbed, it is deposited in the liver as glycogen, and potassium is also stored (see chapter on Potassium Metabolism). Clinical response to parenteral potassium has been noted in a small percentage of patients.

Fisher and co-workers[23] noted that the dumping syndrome could generally be produced in patients affected by the condition, simply by feeding hypertonic glucose. A group under Roberts[21] showed that during the period of dumping, there occurred a fall in the total circulating blood volume. The proposal was put forth that when hypertonic foodstuff reach the jejunum, osmotic forces cause the passage of plasma from the body proper to the gut lumen. This reasoning continues that the resultant decrease in blood volume stimulates pressoreceptors, and this causes the symptoms. The total fall in circulating blood volume is generally under 500 ml, and the change may occur whether symptoms result or not.[13] Hence, it is unlikely that the alteration in blood volume is the cause of the syndrome per se.

The more extensive the surgical resection, as a general rule, the more likely is the occurrence of symptomatic dumping. "Intractable" dumping may occasionally respond to surgical revision of the gastric stump, to produce a narrow outflow orifice. Since symptoms may change, and the condition can improve with time, there are varying criteria for diagnosis. Hence, the reported incidence varies from almost zero to one hundred per cent of gastrectomized patients.

In view of this conflicting evidence, it is not surprising that there is no unanimity of opinion concerning therapy. Oft times a patient will modify his diet so as to prevent symptoms, and the particular medicine he is taking at the time will be credited with the 'cure.' The salient features of diet therapy include frequent small feedings (high protein and low carbohydrate), and lying down after meals. Restriction of fluids during meals may be beneficial; however, the rationale (to reduce the volume) is certainly not easily defended. Although there may be a defect in handling fat after gastrectomy, many of these patients do well when they obtain a large proportion of their calories from lipids.[16]

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# 21

# POTASSIUM METABOLISM*

THE INTIMATE relationship of potassium to gastrointestinal function is of more than passing interest, for it may provide the key to interpreting a number of bizarre clinical manifestations. Indeed, on occasion the recognition and correction of potassium imbalance resulting from gastrointestinal disease, may be of importance in the recovery of a patient. It is the purpose of this chapter to delineate and review the salient features of potassium metabolism as it affects the gastrointestinal tract. The description will, when possible, introduce quantitative terms in order to make the magnitude of the changes more understandable.

## BASIS OF THE INTERRELATION

The importance of potassium to the digestive canal centers about three basic observations.

1. In a healthy individual, daily body requirements of potassium electrolyte are met entirely by intestinal absorption. Abnormalities of absorption may thus deprive the body of its potassium requirement, even though oral intake is "adequate."

2. Potassium normally diffuses across the gut wall in both directions. The "outward" flux, comprising the digestive secretions, often contains potassium in a concentration greater than that of plasma. Loss of this potassium-rich fluid, beyond the amount normally present in the fecal excretion, results in potassium deprivation.

3. Intestinal motor function, and perhaps certain absorptive

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processes as well, are sensitive to the potassium concentration of the internal environment. This apparently works cyclically; deranged gastrointestinal activtiy may result in potassium loss, and the gut deprived of potassium reveals further abnormalities.

## INTAKE

A wide latitude in the quantity of ingested potassium is permissible in the adult; in the presence of renal impairment[1] or parenteral potassium administration[2] this no longer holds true. It has been estimated that 0.22 per cent of the body (or 3950 mEq.) is composed of potassium.[3] Thus, following an average daily oral intake of 3.0 Gm (77 mEq.), the alimentary canal is presented with a load equal to 1.9 per cent of total body potassium.

When radioactive potassium ($K^{42}$, half-life 12.5 hours) is administered, the isotope dilutes itself in a body pool termed "exchangeable potassium." This pool is less than the total store of potassium, since not all of the cation within the body exchanges with the isotope during the short time interval of the experiment. Values for exchangeable potassium in the human male average about 45 mEq. per kg. of body weight.[4] A 70-kg. man has approximately 3150 mEq. (45 mEq. x 70) of exchangeable potassium. If 90 per cent of the daily intake of 77 mEq. were absorbed by the gut, 70 mEq. would be available for dilution in the potassium pool. Each day an adult thus absorbs a quantity equal to 70 mEq./3150 mEq., or 2.2 per cent of the total exchangeable potassium. In steatorrhea, with fecal potassium loss, the level of body exchangeable potassium is greatly reduced.[56]

Intestinal mucosal cells are in a position somewhat similar to the kidney in regard to electrolytes. Facing their external wall is potassium of the gut lumen, usually at a concentration greater than potassium of the extracellular fluid (5 mEq./L.), but below that of the cell proper. Assuming potassium within the intestinal lumen to be 16 mEq./L. (vide infra), and that of the mucosal cell to be 130 mEq./L.,[5] the theoretical energy change involved during the absorption of 70 mEq. potassium from the gut can be calculated. Such a calculation indicates that energy must be expended to accomplish the task.

Actually, suitable account must be made for the electrical gradient across the gut wall (negative with respect to the mucosa),[53] which likely facilitates potassium entry. Maintenance of the electrical gradient is itself an energy requiring process.

Potassium absorption by the gut is thus necessarily dependent upon an energy source (most likely glucose oxidation, as discussed below). Additionally, there is probably an active mechanism for transporting potassium from the blood stream to the mucosa,[6] since large quantities of the element are available for inclusion in alimentary secretions (Figure 13).

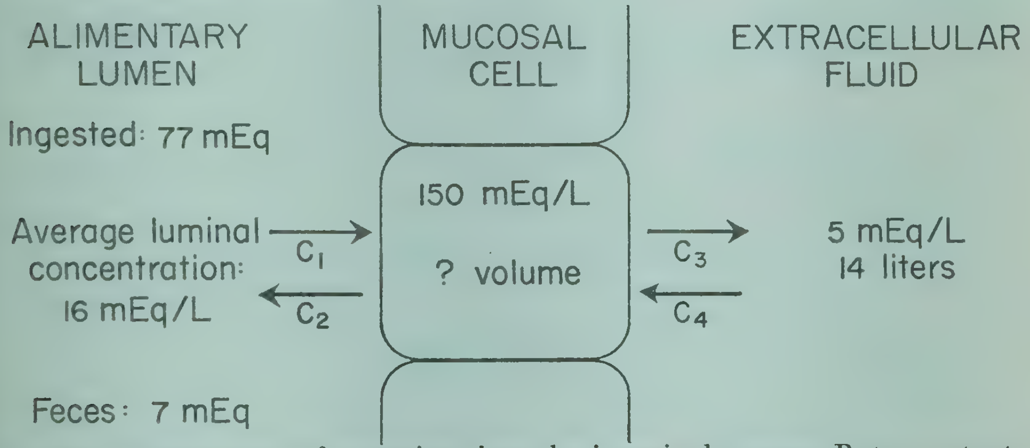
ALIMENTARY LUMEN
MUCOSAL CELL
EXTRACELLULAR FLUID
Ingested: 77 mEq
150 mEq/L
? volume
Average luminal concentration: 16 mEq/L
C1
C2
C3
C4
5 mEq/L
14 liters
Feces: 7 mEq

Figure 13. Movement of potassium into the intestinal mucosa. Rate constants are designated by C and a subscript. Processes indicated as $C_1$ and $C_4$ require an expenditure of energy by the cell.

## INTRALUMINAL POOL

Each of the gastrointestinal secretions contains potassium, but in varying concentrations (Table 11). Potassium within the ali-

TABLE 11

Potassium Content of Alimentary Secretions

| | *Volume of Fluid Secreted (L/day)*[a] | *K Content mEq/L* | *Total K in mEq* |
|---|---|---|---|
| Saliva | 1.5 | 20.0 | 30.0 |
| Gastric Juice | 2.5 | 9.0 | 22.5 |
| Bile | 0.5 | 5.0 | 2.5 |
| Pancreatic Secretion | 0.7 | 5.0 | 3.5 |
| Intestinal fluid | 3.0 | 11.0 | 33.0 |
| Total | 8.2 | | 91.5 |
| Average | 91.5/8.2=11 mEq/L | | |
| Ingested | 2.0 | | 77.0 |
| Grand Total | 10.2 | | 168.5 |
| Grand Average: | 168.5/10.2=16 mEq/L | | |

Values are approximations; reported values differ widely.[2,8,9]

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[a] From Gamble.[7]

mentary lumen is part of the body pool of the cation, and is included in determinations of exchangeable potassium.

This point only recently received adequate attention; there has been recognition that a significant fraction of exchangeable potassium may reside in gut secretions.[10] For example, the rabbit has 7.2 per cent of its exchangeable potassium in the gut lumen. At postmorten, potassium of the human gut lumen is estimated to be 0.44 mEq. per kg. of body weight.[10] Calculations of the volume of fluid in the human gastrointestinal lumen vary, depending upon the assumed concentration of potassium within the secretions. An average value is:

$$\frac{0.44 \text{ mEq./kg. x 70 kg.}}{16 \text{ mEq./L.}} = 1.9 \text{ L.}$$

The value in the living adult may differ from this postmortem estimate.

Human daily fecal potassium excretion averages 7 mEq., but even in normal individuals this will vary widely, depending upon intake. What fraction of the daily excretion is due to newly ingested electrolyte, and what portion arises from body stores, has not been accurately determined. It can be seen that this represents (see Figure 13) a question of calculating the rate at which potassium enters and leaves mucosal cells (a problem amenable to analysis in the analog computer, but incompletely solved at present).

Riklis and Quastel[52] found that glucose absorption from the surviving guinea pig intestine was maximal when the intraluminal potassium concentration was 15.6 mEq/L. This value is remarkably close to the average intraluminal potassium level of 16 mEq/L which we hypothesized (Table 11). The true significance of potassium, within the lumen, in absorption of other substances is being delineated (for instance, potassium migration is related to amino acid transport [54]).

## QUANTITATION OF ABSORPTION

Parenterally administered radioactive potassium can be shown to pass readily from the blood stream to the intestinal cells and thence into the gut lumen. Intestinal mucosal cells are thus presented with potassium both from the daily ingested dose and from their own secretions. To contrast the handling of the potassium load in normal individuals with those having gastrointestinal diseases, a means of measurement is required. Since potassium is taken up by gut cells and "cleared" from the lumen, "clearance" parameters can be defined and evaluated.[11] By the use of such methods, it can be shown that alimentary cells are quite efficient in absorbing potassium (see Appendix). Further studies utilizing radioactive tracers are necessary before the extent of the potassium absorbed and then re-excreted in malabsorption states can be determined. Simple balance studies, while important in delineating the net quantity of potassium in the diet that is not absorbed, tell little of the mechanisms actually taking place at the cellular level.

## FACTORS ALTERING ABSORPTION

Specific control of alimentary potassium absorption is unknown, and several factors are undoubtedly involved. It is not entirely clear whether potassium and other electrolytes are absorbed by gastrointestinal mucosal cells, or whether the portal of entry is via intercellular spaces.[12] The suggestion has been made that electrolytes are carried "passively" as water is actively absorbed; this, however, fails to account for differences in the absorption of sodium and potassium.

### Adrenal Hormones

It is known that adrenalectomy decreases the rate of alimentary absorption of sodium, and to a lesser extent, of potassium.[13,14] There is a concurrent decrease in the ability of the mucosa to take up water and to lower the osmotic pressure of an isotonic solution placed in the lumen. All these defects are corrected by the administration of steroids. Evidence has been presented that desoxycorticosterone, when administered to patients receiving a cation exchange resin by mouth, decreases fecal sodium loss, while it increases the output of potassium.[15,16] Fluorohydrocortisone has approximately the same effect,[17] perhaps indicated that the steroids affect the gut in a manner similar to the kidney.

### Surface-Time Relationship

Absorption requires both a finite time interval and a definite surface area. If either factor is reduced beyond a certain point, some portion of potassium in the diet will not be taken from the lumen. Certain diarrheal illnesses are marked by a rapid transit through the gut. In such cases, the K:Na ratio in the stool becomes numerically greater, as potassium loss in the feces increases.[6] The absorptive failure may perhaps be due to the insufficient time of contact between electrolytes and the mucosa. There has also been recognition that defects of absorption and motility result when the total available surface area of the gut is reduced, such as following surgical extirpation.[18]

### Other Factors

Lack of proper potassium absorption, due to defects of the gut wall, rather than a reduction of its area, are not clearly defined. Net potassium absorption, however, is defective in nontropical sprue and idiopathic steatorrhea,[19,20] as indicated by a large fecal loss and low blood levels. Little information as to the error in these absorptive abnormalities is available; it is conceivable that any or all of the following may be involved—the membrane, the uptake process, or the coupled energy supplying reaction.

The potassium content of blood and tissues is partially dependent upon the pH of surrounding media.[21] Since the acidity or alkalinity of the gut varies over a wide range, local pH variations may have a bearing on potassium uptake. The content of potassium in cellular and extracellular spaces is perhaps also related to uptake of the element. During marked potassium deficiency, the cation is absorbed avidly, without production of an elevated blood level. The potassium concentration of extracellular fluid is greater in infants than in adults,[22] but this has not been correlated with differences in absorption.

Usually, when glucose passes into cells, potassium also enters. An accelerated uptake of carbohydrate from the gut may then carry potassium into the mucosal cells (but not necessarily into the blood stream). In hyperthyroidism there is quickened absorption of carbohydrates,[23] but the velocity of intestinal potassium transport in this endocrinopathy is as yet unknown.

Insulin, which favors the entrance of glucose into tissues, indirectly exerts an influence on potassium transport. During starvation, potassium moves out of cells; in experimental potassium deficiency, a diminution of protein incorporation can be shown to occur, as well as morphologic cardiac lesions.[24,25] Gastrointestinal changes during experimental potassium deficiency are not well defined morphologically, although easily demonstrable functionally.

When present in low concentrations in the gut lumen, potassium may enter the lymphatic supply and interstitial fluid before passing into the blood stream.[6] Thus there is reason to suspect an alteration of potassium absorption when the intestinal lymphatic flow is impaired. It has been reported that lymphosarcoma may involve the intestinal lymphatics, with resultant malabsorption and excessive fecal loss of ingested potassium.[26]

In the final analysis, the rates of cellular absorption and secretion (Figure 13) determine the net uptake of potassium from the lumen. Hence, even though absorption of electrolytes is great in the duodenum and jejunum, the secretory rate is of almost equal magnitude,[27] and the net gain may be small. In the colon, although the transport rate is slow,[6] absorption is greater than excretion, and the net uptake of the element becomes appreciable.

## POTASSIUM LOSS FROM THE ALIMENTARY CANAL

Potassium is continually excreted in both stool and urine, making daily replacements necessary to counter the loss. An individual with an alimentary disorder and reduced oral intake still has need of potassium. Even if a modified intake is provided, the replacement may be insufficient to meet demands.[28] Further, intravenous saline and glucose, so commonly administered when oral nutrients can not be tolerated, may enhance potassium loss via the kidneys[2] as part of the general electrolyte loss during a diuresis.

### Increased Alimentary Output

Potassium may leave the digestive system in excessive amounts as the result of vomiting,[29] suction,[9] fistulae,[9] use of cation-exchange resins,[30] use of enemas,[28,31] chronic use of cathartics,[32,33] diarrheal illnesses,[6,55] malabsorption,[19,20,26] and loss of blood and tissue.

As a result of deprivation of potassium in the alimentary lumen, other sites are depleted. The net result may be a pan-tissue deficit, with such accompanying alterations as hypokalemia and electrocardiographic abnormalities; such studies may not, however, exactly correlate with tissue potassium stores.[29]

An interesting example of a pan-tissue potassium lack has been reported by Schwartz and Relman.[32,33] The condition arose in a small group of individuals who chronically used cathartics and suffered from loose stools. The morphologic change in the kidney, observed following renal biopsy, reverted toward normal when potassium was administered.

## RELATION OF OTHER DEFECTS OF POTASSIUM TO ALIMENTARY FUNCTION

### Dumping Syndrome

This complex includes postcibal tachycardia, sweating, and pallor; it is occasionally seen in individuals who have had a major portion of their stomach removed. The effect is apparently a sympathetic response, although the mechanism of production is unknown. In a number of cases of the dumping syndrome, clinical improvement has been reported to follow the oral administration of potassium.[34,35] The suggestion has been put forth that glucose "dumped" into the small intestine is rapidly absorbed, and that potassium enters the cells simultaneously. An extracellular deficit of potassium, and symptoms, may then occur.

### Ureterosigmoidostomy

Implantation of the ureters into the bowel has been used when the bladder must be bypassed. The entrance of urine into the rectosigmoid produces aberrations in serum electrolytes, although preceding renal disease may contribute to the abnormalities. Excess chloride replaces bicarbonate from the blood stream. Frequently associated with this hyperchloremic acidosis is a depression of blood potassium levels. Produced by a mechanism still undefined, the hypokalemia may be severe enough to result in muscle weakness and quadriplegia.[36] There is also evidence of a carbohydrate disturbance in ureterosigmoidostomy;[37] perhaps potassium follows glucose into cells, depleting extracellular fluid of the electrolyte.

### Familial Periodic Paralysis

Most patients with this syndrome have depressed blood levels of potassium during an attack of muscle palsy or paralysis. Evidence has been presented that variations of aldosterone levels may be involved.[38] Typical attacks of the syndrome, including hypokalemia and muscle involvement, can often be induced by ingestion of a high carbohydrate meal[39] (perhaps again due to potassium following carbohydrate into cells, depleting the extracellular fluid) . Painter[40] reported a case of familial periodic paralysis showing a diabetic glucose tolerance curve during a low potassium episode. After several days of potassium administration, a repeat test was normal.

The relationship of potassium to the absorption of other materials is unknown, although in the frog, potassium increases the permeability of the intestine to glucose.[41] The functioning of the gut during hypokalemic episodes has not been completely studied. Adequate data to support or refute the supposition that intestinal mobility diminishes during low potasium episodes of the syndrome, are not available. Gass and associates[39] presented a patient with familial periodic paralysis who had only two bowel movements during the six days of a low potassium episode. Recently, an entity has been described which clinically resembles familial periodic paralysis but is characterized by elevated blood potassium values;[42] information as to intestinal activity in this disease state is also lacking.

## FUNCTION OF POTASSIUM IN ALIMENTARY CELLS

It is usually recognized that alimentary disorders may result in potassium alterations. Recognition of the converse, namely that alterations of the potassium level can lead to gastrointestinal derangements, is not as readily made clinically. Experimental evidence has accrued that a deficiency of potassium may induce intestinal paralysis.[43,44] Clinical data are also available that postoperative intestinal distention may follow potassium loss.[45,46] Schlesinger reported a correlation between abdominal distention and potassium deficiency.[47,48] A blood potassium level of 3.1 mEq./L. was critical; abdominal distress with vomiting and distention often followed when lower levels were present.

Three principal actions are probably performed by potassium within the alimentary apparatus.

1. Potassium is the principal intracellular cation of both the mucosa[5] and surrounding muscle cells.

2. Alimentary secretions contain potassium,[2,8,9] and an active mechanism is perhaps involved in transferring this cation from the blood stream to luminal secretions.[6]

3. Potassium plays a role in the functioning of nerve and muscle cells within the gut wall. The digestive canal is essentially a hollow tube, surrounded by three muscle groups (circular, longitudinal, and mucosal). Alimentary smooth-muscle cells function in a manner fairly similar to other smooth-muscle groups, and they are sensitive to changes in potassium concentration.[49] Born and Bulbring[50] demonstrated that potassium moved out of alimentary muscle cells following stimulation. There are also observations that potassium loss in general can deprive gastrointestinal muscles of this element,[43,44] perhaps accounting for distention and ileus.

## THERAPEUTIC ASPECTS

An awareness of the importance of potassium in gastrointestinal disorders will frequently lead to the recognition of disturbances involving the electrolyte. Hypokalemia is an entity of great clinical urgency, for it has been shown that such patients have a mortality rate three times greater than the general hospital population.[51]

If any of the causes of potassium loss, or reduced intake, have been present, suitable studies are in order. Aside from determining the blood potassium level, the serum bicarbonate value as well as the electrocardiogram may aid in establishing the diagnosis. However, such studies often fail to exactly correlate the tissue potassium levels. Because of this, provided the patient is hydrated[48] and no renal disease is present, administration of potassium may be indicated when a deficit is suspected. Large amounts of the cation may be required when pan-tissue potassium deficiency is present. Therapy should continue until a major portion of cellular potassium is replaced. The exact quantity to administer is difficult to determine. Vigorous therapy can be tried initially since the patient with reduced potassium stores is in grave danger. After the initial measures, a more cautious attitude is needed, with continuing observation.

Whenever possible, potassium should be given orally. If administration by a stomach tube is not feasible, the subcutaneous route may be used. Only when these two techniques fail to meet the patient's needs should the intravenous route be used for potassium input. The danger of potassium overconcentration during therapy is unlikely, provided the intravenous path is avoided. In cases of hyperkalemia, saline and glucose to flush out potassium,[2] calcium to counteract nervous system effects, and insulin to send the cation intracellularly often suffice in taking the patient past the immediate danger.

Mention must be made of the digestive tract during hyperkalemic episodes of systemic diseases. It is occasionally forgotten that the alimentary canal offers easy access to potassium stores, both in the normal individual and in cases of uremia. Indeed, during uremia, the gut wall contains more potassium than normal.[14] An enema can flush out as much as 87 mEq. of potassium per liter;[28] lavage of the digestive tract may on occasion, tide a uremic patient over an acute episode, without having to resort to artificial dialysis.

## SUMMARY

The relationship of potassium to gastrointestinal function has been discussed, and quantitative expressions have been introduced in areas where such data are available. On an average diet, 77 mEq. of potassium are ingested, and 7 mEq. excreted in the feces. The daily absorption amounts to 2.2 per cent of exchangeable potassium.

Potassium is transported in both directions across the intestinal mucosa. Average concentrations of potassium are: gastrointestinal contents 16 mEq./L., mucosal cells 130 mEq./L., and extracellular fluid 5 mEq./L. An energy change involved in potassium absorption and factors influencing absorption have been described. Quantitative aspects of potassium uptake and excretion by the gut have been presented (Appendix), and the mucosal cells have been shown to be efficient in their handling of the cation. Other diseases which alter potassium levels and hence influence gastrointestinal function have been discussed.

The clinical significance of altered potassium stores in terms of gastrointestinal function has been pointed out and the therapeutic aspects discussed.

## APPENDIX

### Determination of Potassium Clearance

The simplest parameter that can be evaluated is the percentage of the daily dose that appears to be absorbed.[11] Such a value is "static" in that it gives little insight as to the workings of mucosal cells or potassium turnover. It simply indicates how much of the intake appears in the stool. Hence, "static clearance" ($C_s$), the percentage absorbed, may be defined in terms of the quantities ingested ($Q_i$) and excreted ($Q_e$):

$$C_s = \frac{Q_i - Q_e \times 100}{Q_i}$$

For a homeostatic individual (77 mEq. ingested, 7 mEq. excreted), $C_s$ is 90 per cent. In a diarrheal illness, if 21 mEq. appeared in the stool, $C_s$ would be 73 per cent.

Static clearance, however, neglects the quantity of potassium brought to the luminal surface by gastrointestinal secretions. In addition to the ingested cation, mucosal cells have to contend with potassium in the alimentary out-pourings. Taking this additional amount ($Q_p$, the quantity presented) into consideration, we can arrive at a more dynamic or "active" evaluation of the mucosa's handling of potassium. Thus, active clearance ($C_a$) is:

$$Ca = \frac{(Q_i + Q_p - Q_e) \times 100}{(Q_i + Q_p)}$$

It will be seen that this equation differs from the preceding one only by having the quantity (ingested) replaced by (ingested plus presented by secretions). From Table 11, an approximate value for the total potassium content of the alimentary secretions can be obtained (91.5 mEq.). Employing this value for $Q_p$, "active clearance" can be determined; in the normal individual this is 96 per cent. To phrase it another way, mucosal cells appear to be 96 per cent efficient in absorbing the total potassium load presented at their luminal surface. In the diarrheal state mentioned, if 21 mEq. were lost in the stool while other secretions remained unchanged, $C_a$ would be 87 per cent (compare this value with the 73 per cent determined by use of static clearance). It is thus apparent that, in order to speak of the efficiency of mucosal cells, full recognition must be made of the materials present in the alimentary secretions, which also vie for absorption.

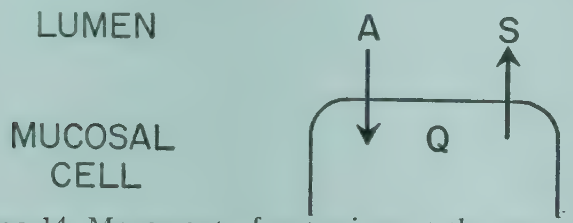

Figure 14. Movement of potassium at the mucosa-lumen interface. At homeostasis: $Q + A - S =$ Constant, where Q is the quantity within the cell, A is absorption, and S represents secretion.

Using the nomenclature of Figure 14, the ratio of absorption to secretion at the mucosa-lumen interface may be represented by A:S. This fraction is designated the "clearance gradient" ($C_g =$ absorption over secretion), since it offers a means of evaluating the ability of mucosal cells to absorb or clear potassium in the presence of secretion of the electrolyte. The clearance gradient may be evaluated by means of the equation:

$$Cg = \frac{(Q_i + Q_p) - Q_e}{Q_p}$$

For the homeostatic individual, $C_g$ is 1.7. That is, 1.7 times more potassium is absorbed by the gastrointestinal tract, than is secreted into its lumen.

Total activity of mucosal cells in transporting potassium both ways (absorption and secretion) across the cell boundary is simply the sum of A + S. The total clearance ($C_t =$ absorption plus secretion) can be defined in terms of: $C_t = (Q_i + 2Q_p) - Q_e$.

Again employing the values discussed, $C_t$ is 253 mEq. per 24 hours. Only 77 mEq. were ingested, but at the luminal surface, mucosal cells handled 253 mEq. of potassium (3.3 times the amount taken orally). The magnitude of this figure makes it more readily understandable why even a slight defect in absorption will result in potassium wasting by the gut.

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# 22

# PROTEIN LOSS

THERE IS GENERAL recognition that electrolytes are present in alimentary secretions, and that loss of such fluid depletes body electrolyte stores. It is the purpose of this chapter to emphasize that gastrointestinal fluids also contain considerable amounts of protein; hence, removal of digestive secretions may be associated with protein depletion. Since proteins exiting from the alimentary canal can originate not only from secretions, but also from ingested food, blood, and tissue sloughed from the wall, a discussion is given of the differential diagnosis of protein loss from the digestive apparatus.

## CRITERIA FOR DETERMINING FECAL PROTEIN LOSS

The occurrence of excessive fecal protein loss can be demonstrated by five procedures, one or more of which may be applicable to a particular case under study.

1. *Increased fecal nitrogen.* Chemical demonstration of elevated fecal nitrogen is the most widely applicable test, but is only presumptive evidence of excessive protein loss. The evidence is only presumptive since it is tacitly assumed that a normal diet has been ingested (without an excess of nondigestible nitrogenous materials). Over a wide range of intake, nitrogen excretion in the feces is independent of the amount of nitrogen in the diet[18] (providing it is digestible). Since normal excretion of nitrogen in feces is independent of dietary intake, average values can be given; representative figures are 1.0 ± 0.5 grams per day[1] and 1.7 ± 0.8 grams per day.[2] About one half of this nitrogen is accounted for by bacteria. Whether administration of nonabsorbed antibiotics, which destroy much of the intestinal flora, is followed by altered

fecal nitrogen excretion is uncertain. The remaining half of fecal nitrogen arises from intestinal secretions and mucous, desquamated cells, intact and degraded enzymes, blood and exudates in pathologic states, and food residues (this component normally accounts for less than one-tenth of fecal nitrogen excretion).

2. *Presence of food muscle fibers in feces.* This piece of evidence is the most easy to demonstrate and yet most rarely found. Muscle proteins are usually readily digested and hence their increased presence in feces (demonstrated microscopically) is definitive evidence of abnormal protein loss. Indeed, all proteins in the average diet are enzymatically degraded with ease except some plant proteins which may be enmeshed in cellulose, and collagen fibers which are indigestible while 'raw' but become more digestible after cooking.

3. *Increased radioprotein in stool.* Loss of ingested proteins via the feces has been studied by means of radioactive tracers. To facilitate detection, a protein is usually tagged with an isotope which emits gamma rays; $I^{131}$ (half-life 8.1 days) is commonly used for this purpose. Since proteins are digested in the gut and the resulting amino acids are absorbed across the intestinal mucosa, little of the radioactive tag appears in the feces. An increase in fecal radioactivity indicates either impaired digestion or malabsorption. Serum albumin-$I^{131}$ is the most commonly employed substrate, and variants of the methodology have been described.[3] Iodine hydrolyzes from the protein after the material has been stored for a period of time, and the 'shelf life' is limited.

4. *Demonstration of blood proteins in stool.* Blood proteins do not occur in the intestinal lumen in significant quantities. Hence, their demonstration in alimentary fluids or stool indicates either ingestion of blood or its passage from the blood stream to the digestive lumen.[4] Two types of procedures are used to show the presence of blood proteins in the gut lumen: the occurrence of labeled protein in the lumen following its intravenous injection, and the immunological or physical separation of specific proteins. Thus $I^{131}$-proteins administered intravenously can be found in the intestinal contents of certain patients with regional enteritis and ulcerative colitis, indicating a 'leak' across the wall.[4] Specific antibodies

also detect distinct blood protein components in the luminal material of these patients. Protein leakage also is demonstrable in giant hypertrophy of the gastric mucosa.[5] Such sources of protein removal from the body can be severe enough to result in hypoproteinemia and a diminution of the albumin pool. Normally about 360 grams, the albumin pool (determined by the distribution of tagged albumin) may be as low as 120 grams due to prolonged fecal loss and inadequate replacement.

The ropy intestinal material in meconium ileus, associated with cystic fibrosis of the pancreas, has been shown to have a high content of serum albumin.[6] The mechanism whereby albumin passes into the gut lumen in this disorder is unknown. A leakage of serum proteins into the alimentary lumen has also been demonstrated in radiation damage.[7]

5. *Blood and sloughed tissue in feces.* The occurrence of these products in stool is evidence of abnormal protein loss since they are not usual constituents of fecal material.

## PROTEINS OF ALIMENTARY SECRETIONS

Even in the absence of disease states, alimentary secretions contain considerable amounts of proteins (which may be classified according to their physical behavior [8]). Table 12 summarizes data from the literature concerning the protein content of digestive secretions. From the table it can be seen that pancreatic fluid is much richer in proteins than other alimentary liquids. In an average adult, a total of some 35 grams of protein is secreted into

TABLE 12

PROTEIN CONTENT OF ALIMENTARY FLUIDS

| *Fluid* | *Reference* | *gm Protein/100 ml Secretion* | *ml Secretion in 24 Hours*[15] | *Total Protein Content in 24 Hours* |
|---|---|---|---|---|
| Salivary # | 10 | 0.30 | 1,500 | 4.5 |
| Gastric* | 11 | 0.37 | 2,500 | 9.3 |
| Biliary** | 12 | 0.20 | 500 | 1.0 |
| Pancreatic | 13 | 1.50 | 700 | 10.5 |
| Intestinal* | 14 | 0.33 | 3,000 | 9.9 |
| | | | Grand total: | 35.2 gm/day |

# Average; range is given as 200-400 mg/100 ml.

* Obtained by multiplying nitrogen content by 6.25.

** 'Mucin' content given as 0.8-2.8 gm/liter; other proteins may also be present.

the digestive canal per day. The quantity of secretion varies with the diet so that the amino acid mixture in the lumen, ready for absorption, is approximately constant.[16] In tropical sprue,[17] and perhaps in other disorders, the nature of the secreted proteins is altered.

Three sequelae of the secretion of protein into the lumen can be noted.

1. Since stool loss of protein in usually negligible, we can infer that secreted proteins (or their digestive products) must be almost completely reabsorbed. This is true even when the daily protein intake is greatly increased.

2. Lumen cells handle not only the 60 to 120 grams of protein in the daily diet, but also the additional 35 grams of secreted protein. An absorptive error by these cells may thus result in the loss of both ingested and secreted protein.

3. Removal of fluids during intubation of the alimentary canal may result in the loss of many grams of protein per day. When this loss is added to the absent oral protein intake of intubated patients, the rapid fall in body protein stores becomes understandable. Proper therapy must replace fluid, electrolytes, and proteins.

The loss of alimentary fluids by any route carries away protein. Patients with prolonged vomiting, excess mucous secretion ("mucous colitis"), or fistulae should be suspected of having protein depletion.

## DIFFERENTIAL DIAGNOSIS OF PROTEIN LOSS FROM THE ALIMENTARY CANAL

Listed in Table 13 are the principal conditions associated with protein loss from the digestive system. Aside from an excessive loss of alimentary fluid, four large diagnostic groupings remain to account for excess fecal protein: altered digestion, impaired absorption, leakage across the mucosa, and sloughing of blood and tissue.

The establishment of a specific diagnosis by standard procedures usually explains the source of proteins in stool. Impaired protein digestion is almost always due to a pancreatic defect; the stool, or duodenal fluid, may be examined for pancreatic enzymes in order to establish the competence of this organ's exocrine function. Normal digestion with accompanying malabsorption occurs in a number of

TABLE 13

CAUSES OF PROTEIN LOSS FROM THE GASTROINTESTINAL TRACT

1. Loss of alimentary fluid
    a. Mechanical removal of secretions
    b. Vomiting
    c. Diarrhea
    d. Fistulae
2. Loss in feces due to impaired digestive enzymes
3. Loss in feces due to impaired absorption
    a. Malabsorption syndromes
    b. Conditions causing a rapid transit time
4. Loss in feces due to protein leak across mucosa
    a. Hypertrophy of gastric mucosa
    b. Certain cases of regional enteritis and ulcerative colitis
    c. Radiation damage
    d. Meconium ileus (?)
5. Loss of blood and tissue in feces
    a. Infestations
    b. Ulcerative lesions

disorders. Absorption studies can be helpful in these cases, but it should be remembered that one type of nutrient may be normally absorbed while others are not well taken up from the lumen. The actual course of the disorder may be followed by clinical observation and chemical determinations of fecal nitrogen.

Protein loss in feces, due to leakage across the mucosa, should be suspected when any one of the five major causes is present (hypertrophy of the gastric mucosa, certain cases of regional enteritis and ulcerative colitis, radiation damage, and meconium ileus). The demonstration of blood proteins in stool by means of antibodies, or the detection of parenterally administered radioprotein, has already been discussed.

The loss of blood and tissues from the alimentary canal can also result in protein depletion. Stool guaiac studies will give a qualitative estimation of the magnitude of blood loss and a quantitative determination can be made by using red cells tagged with radiochromium[9] (vide infra).

## PRACTICAL METHODS FOR QUANTITATING PROTEIN LOSS

A simple and approximate method for estimating protein loss in fluids removed by intubation is to observe the volume and then use the values for protein concentration per 100 ml given in Table 12. Another procedure is to pool all the secretions and use the fundatmental relationship:

$$\text{Weight protein} = \text{weight solution} - (\text{weight electrolytes} + \text{water}).$$

The total weight of water can be roughly obtained from direct observation of the volume (since water weighs 1 gm/ml). Electrolytes can be approximately assumed to contribute 0.9 gm/100 ml. Precise values for the proteins in removed secretions may be obtained by chemically analyzing for total protein or nitrogen content. Since more accurate figures contribute nothing to the management of a particular case, these tedious processes are not worth the effort.

Fecal nitrogen determinations may be helpful in following the course of a malabsorption state, but the procedure takes time. Blood loss can be quantitated by intravenously administering labeled erythrocytes and subsequently estimating radioactivity in the stool (due to the presence of the tagged cells). The actual blood protein loss may then be calculated from the identity:

$$\text{grams protein/100 ml blood} = \text{grams hemoglobin/100 ml} + \text{grams serum protein/100 ml}$$

Normally 100 ml of blood contains 15 grams of hemoglobin and 7 grams of serum proteins (a total of 22 grams of protein). Tissue sloughing also carries away large amounts of protein. Alimentary tissue is approximately 70 per cent water and 30 per cent solid material. Of the solid matter, two-thirds is protein. Hence the loss of 100 grams of tissue removes 20 grams of protein from the body. Bleeding and tissue necrosis in ulcerative colitis is thus associated with considerable protein loss.

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# 23

# MALABSORPTION

In the strictest sense, malabsorption applies to the diminished, or tardy absorption of materials from the gut lumen. On this basis, the single adequate diagnostic criterion is the rate of disappearance of a test material from the lumen. Absorption, however, is a complex process which involves at least three steps: passage from the lumen to mucosal cell, processing or storage in the cell, and discharge into the lymphatics or blood stream. Thus, in the broadest interpretation, malabsorption includes any dysfunction of intestinal cells which involves delayed uptake, defective conversion, prolonged storage, or even bacterial destruction of the material under study. This point is essential, since certain test substances (as vitamin A) may be normally taken from the lumen into mucosal cells, but stored for varying periods before being passed into the blood stream[1] (the stimulus for discharge may be vigorous contractions of the gut). The tardy appearance of an ingested metabolite in the blood stream is perhaps not sufficient evidence per se to definitely state that a defect exists at the lumen-mucosa interface. Further, absorption studies must be viewed with caution since certain combinations of nutrients may interfere with each other's uptake,[2,3] creating an impression of malabsorption. It is also of importance to recognize that malabsorption is simply a designation for a disordered process, and use of the term implies nothing as to the underlying disease, the extent of the defect, or the nutrients affected.

Reviews discussing tropical sprue,[4] non-tropical sprue,[5] and the malabsorption syndrome in general,[6] may be consulted for clinical descriptions of the diseases. The initial difficulty encountered is that there is no uniformity as to the criteria proposed for diagnosis of these disorders. Indeed, the availability of devices for obtaining intestinal biopsies during life,[7,8] has provided evidence that some clinical subdivisions are not justified, as they represent different stages of the same process (that is, certain types of sprue, celiac disease, and 'idiopathic steatorrhea' may be the same disease viewed at different times [9,10]).

The etiology of 'idiopathic' malabsorptive disorders is unknown. Principally emphasized have been nutritional deficiencies, infection, abnormal metabolites (rancid fats),[4] and genetic factors.[10]

### Nutritional Deficiencies

There is a high incidence of malabsorption among certain groups, in semitropical areas, who eat a marginal diet. While it is conceivable that a dietary deficiency may be the cause of the disorder, it is equally likely that the deficiency merely acts as a trigger to accentuate a condition already present. That is, the most striking effects of malabsorption are due to lack of various nutrients in the body; if inadequate intake is added to inadequate absorption; symptoms may be precipitated. The therapeutic value of vitamin $B_{12}$ in pernicious anemia, on the other hand, indicates not a deficiency of the vitamin in daily diets, but rather a mucosal error which prevents its absorption during the disease. Folic acid has been used in the treatment of tropical sprue, often with good results. However, on the basis of what we have said, this can not be interpreted to mean that a deficiency of folic acid necessarily causes the malabsorption. The suggestion has been made that undue exposure of food not only destroys its nutritive value, but also converts some of its fats to noxious products[4] which damage the mucosa. The detrimental effect of wheat gluten on certain cases of malabsorption suggests a toxic factor, rather than a deficiency, in the genesis of the altered uptake. The difficulty with such speculation, however, is that we are uncertain that we are dealing with a single disease entity.

### Infections

We must state at the outset that no differences in the bacterial population of the alimentary tract (as regards species, numbers, or distribution) have been detected in cases of primary malabsorption (see chapter on Intestinal Bacteria). However, several observations have suggested that infection may play a role in the genesis of malabsorption. There can usually be elicited, from a small percentage of afflicted patients, history of an antecedent bout of bacterial or amebic dysentery. Clinical improvement of cases of malabsorption may follow use of sulfonamides[79] or broad spectrum antibiotics.[80] Such results can also be interpreted to mean that normal bacteria utilize metabolites needed for absorption, but not present in sufficient quantity in the diet. Patients with certain errors of intestinal absorption have been shown to excrete skatole derivatives which are apparently produced by bacterial action on the amino acid tryptophan.[112,113] After antibiotic treatment, excretion of these products diminishes; whether this parallels clinical improvement is uncertain. Recently the excretion of indoleacetic acid (also a tryptophan derivative) has been shown to be depressed by the administration of neomycin to patients with malabsorption.[109]

### Genetic Factors

The distribution of malabsorptive disorders among individuals does not permit a statement as to a clear cut type of Mendelian inheritance. However, the disease seems to 'run' in families, and may be associated with a higher incidence of blood type O than normal.[11] In addition, intestinal histologic changes in childhood celiac disease closely resemble those of adult sprue,[10] suggesting that the defective pattern, whatever its nature, is determined early in life. It can be noted that these conclusions are highly tentative, and additional data are sorely needed.

### 'Secondary Malabsorption'

In most cases of malabsorption noted in the temperate climate of North America, the defective uptake will prove to be secondary to a recognizable basic disease process. Certain of these entities are well recognized, while others are only now being identified. Among the 'secondary' malabsorptive disorders are pancreatic and gastric diseases which reduce the influx of digestive enzymes or interfere with their mixing with food. In a sense, these are errors of digestion and not of absorption. True secondary malabsorptive errors can be noted in instances of blockage of intestinal lymphatic channels (as during the course of intestinal lymphoma and Whipple's disease), anoxia of the intestine induced by occlusion of mesenteric vessels by thrombi or emboli,[18,19] surgical removal of part of the absorptive area, and operative creation of a blind loop of intestine (a cause of malabsorption which is often remedied by use of antibiotics [16]).

Since the cause (s) of the error is unknown, there is no uniformity as to diagnostic criteria or therapeutic approach. I am of the opinion that attempts to set up arbitrary criteria of classification based on the uptake of specific metabolities[12,13] are unwise; the absorption of any substance may be impaired in a specific case (although fats are most commonly involved [14]). No classification is entirely adequate, but that listed in Table 14 has been found useful. Our thinking in terms of malabsorption has become stereotyped and limited to entities which result in diminished uptake of nutrients. More generally though, malabsorption also includes

TABLE 14

A CLINICAL CLASSIFICATION OF THE DISORDERS RESULTING IN MALABSORPTION

| | |
|---|---|
| 1. *Anatomic defects.* | Gastrectomy[15] |
| | Fistulae |
| | Diverticulosis[16] and 'blind loop' |
| | Enteroenterostomies |
| | Congenital errors |
| | Lymphatic obstruction[20] |
| | Intestinal infarction[18,19] |
| | Resection of intestine[17] |
| 2. *Enzymatic defects.* | Pancreatic exocrine dysfunction |
| | Diminished enzyme accessory factors (as bile delivery) |
| 3. *Pathologic damage to intestine.* | Irradiation injury |
| | Neoplasm |
| | ? collagen disease of intestine[98] |
| | Infections and infestations |
| | ?Dystrophica myotonica[26] |
| | Whipple's disease |
| | ? Acrodermatitis enteropathica[25] |
| | Other intestinal insults |
| 4. *Metabolic disturbances.* | Thyroid, adrenal, other endocrine disorders |
| | Malnutrition and Kwashiorkor |
| | Avitaminosis |
| | ? Allergy |
| | ? Hypertonia and rapid transit |
| 5. *Primary or idopathic disorders.* | Sprue (tropical, non-tropical, celiac disease) |
| | Hemochromatosis |
| | Wilson's disease |

supra-absorption (greater uptake than normal). Because of this consideration, hemochromatosis (excess iron absorption) and Wilson's disease (excess copper absorption) have been included in the table under the idiopathic classification, and are more fully discussed in the following chapter.

## DIAGNOSIS

A detailed history and physical examination will often serve to establish a diagnosis of malabsorption. If these are the only techniques employed, however, many cases will be missed and adequate standards will not be available for following the course of the disease. Family history, childhood affliction by the celiac disorder, type of diet and nutritional supplements, pain and abdominal complaints, type and frequency of bowel movements, history of residence in the tropics, and past alimentary surgery should all be noted. Flatulence, intolerance to fatty foods, weight loss, tetany, and aphthous stomatitis ('sprue') are all valuable diagnostic clues. This is the first stage of the diagnostic work-up which is summarized in Table 15.

TABLE 15

An Approach to the Evaluation of Malabsorption Disorders

1. History
2. Physical examination
3. Stool and blood examination
4. Tolerance and balance tests
5. Biopsy: oral mucosa, rectal mucosa, intestinal mucosa
6. X-ray studies of the intestinal tract & accessory organs
7. Surgery as indicated for lymph node biopsy or exploratory laparotomy
8. Therapeutic trial
    A. Pancreatic and biliary supplements
    B. Agents to decrease motility (?)
    C. Antibiotics
    D. Vitamins
    E. Gluten-free diet
    F. Steroids

If malabsorption is secondary to another process, the basic disease may leave its tell-tale marks (about 80 per cent of patients with malabsorption, in North America, have the secondary type). Physical findings depend upon the length of time the disorder has been present. The classification of tropical sprue proposed by Stefanini[21] as modified by Gardner[4] is helpful in orientating our thinking along this line. In the first stage of the disease, there is fatigue, bulky bowel movements, and often little else. Weeks to months later, continued malabsorption has depleted nutrients and resulted in weight loss; most of the signs and symptoms are related to one or more nutritional deficiencies. In the third stage, frank deficiency is present, muscle wasting occurs, and macrocytic anemia can be found.

Well developed cases of idiopathic malabsorption suffer from cachexia and asthenia, and often have pale melanotic pigmentation on exposed areas of the body (the relationship of dermal pigmentation to gastrointestinal disorders is discussed in a separate chapter); in Whipple's disease, pigmentation may also be prominent. The pigmentation and asthenia are strongly suggestive of Addison's disease, although frank adrenal malfunction has not been proved in the malabsorption syndromes. Pigmentation has also been noted in the intestinal musculature of patients with malabsorption,[22] a location quite different from that in melanosis coli.

Abdominal distention with relatively little tympany can be present in idiopathic malabsorption; this is due to the presence of fluid in the intestinal lumen.[23] Liquid in the bowel loops results from diminished absorption of water and electrolytes[24] (which may be an important manifestation of the illness), and again suggests a relationship to Addison's disease. The Kepler-Powers water test can reveal an increased nocturnal urinary volume,[29,30] a finding also observed in adrenal hypofunction. The nocturnal diuresis may simply be the result of delayed absorption of fluids taken during the waking hours, or it may be a mechanism designed to aid uptake from the lumen (this of course is conjectural).

Aside from the pigmentation of intestinal muscles in certain cases of idiopathic malabsorption,[22] there are other interesting muscle abnormalities. Dystrophica myotonica[25] in an adult has been reported, in association with a sprue-like syndrome. In the late stages of malabsorption, there is muscle wasting, with concomitant creatinuria.[27] Part of the muscle difficulties are probably related to the loss of electrolytes (particularly potassium)[48] and it is known that potassium deficiency of a severe degree may result in intestinal paralysis.[28]

The weakened abdominal musculature and fluid filled intestinal loops produce a 'pot bellied' appearance in far advanced cases of primary malabsorption. Skin changes reflect vitamin deficiencies and poor nutritional status. Peripheral edema as a manifestation of hypoproteinemia is far more common than frank anasarca in these patients. The finding of lymphadenopathy should suggest lymphoma or other underlying disease as the basis of the malabsorption. Lymphadenopathy, however, also occurs in Whipple's disease.

Early in the course of malabsorption, there is little change in the blood picture. Later, however, mild disturbances and even macrocytic anemia may occur (sometimes complicated by iron deficiency). The development of blood dyscrasias may be a reflection of as yet unknown factors, but also is due to the lack of transport of vital nutritional factors across the gut mucosa. This has been called a form of starvation in the midst of plenty, or a "conditioned deficiency disease." [31] In pernicious anemia, for example, there is virtual isolation of the affected person from the vitamin $B_{12}$ in his gut lumen.[32] This is characterized by inability to transport a millionth of a gram of vitamin $B_{12}$ per day from the alimentary lumen to the blood stream. Blood examination may also reveal a severe hypoproteinemia[33] (often below 2 gm/100 ml); this is the probable explanation of peripheral edema and the bleeding tendency (due to low prothrombin [34]). Another significant finding is a depressed serum level of carotene, frequently below 40 micrograms/100 ml (normal = 60 to 200). Anemia, hypoproteinemia and hypocarotenemia, are likely the result of impaired uptake of essential nutrients. Blood electrolytes are more or less deranged depending upon the degree of severity of the illness. Hypocalcemia can accompany a low blood protein level, or may be an independent finding; tetany in the course of a malabsorption illness should direct attention to the blood calcium level, although potassium and magnesium imbalance can perhaps be contributory factors. Measurement of body potassium which is exchangeable with injected radioactive $K^{42}$ reveals a depletion of potassium stores in patients with malabsorption.[48]

Stool examination can sometimes be a helpful procedure. A greasy and bulky specimen immediately suggests steatorrhea. Staining the feces with sudan III or IV (or other fat stains)[102] serves to make neutral fat droplets prominent on microscopic examination (but soaps do not take the stain). Mineral oil taken prior to the test invalidates the result, since the oil also stains. At best, the tests are qualitative and must be supplemented by other procedures.

Some clinics have placed importance on the presence of undigested starch in the feces as an indication of 'starch intolerance,' a supposed variant of the malabsorption syndrome. Evidence for this is very meager. Rather uncritically, the addition of iodine to stool specimens (iodine and starch produce a blue color) has been used as a clinical laboratory test. Davidson and Bauer[35] have criticized this procedure, noting that 43 per cent of casually passed stool specimens contained extracellular starch on microscopic examination. They also reported that the use of enemas, and bowel 'sterilization' by neomycin, increased the starch content of feces. Hence, the conclusion was reached that the iodine test for extracellular starch in feces was not a valid indication of 'starch intolerance.'

In 'secondary' malabsorption, due to infestation, examination of stool may reveal the presence of ova and parasites. It is well recognized that the presence of Diphyllobothrium latum (fish tape worm), in the duodenum, can result in a clinical picture resembling pernicious anemia (see section on Infestations). Giardia lamblia infestation has, uncritically, occasionally been claimed to result in an altered motility pattern and malabsorption. Tuberculosis of the intestine can be accompanied by decreased uptake from the lumen, but the specific etiologic diagnosis is rarely made prior to laparotomy.

A discussion of tolerance and balance studies will be deferred a few paragraphs until after mention of other steps in the diagnostic evaluation. Biopsy studies have proven of considerable value in recent years in diagnosing the malabsorption syndrome. Scrapings of the oral mucosa may reveal abnormal cellular patterns. Some workers have interpreted this as indicating that the intestinal manifestations of primary malabsorption syndromes are but part of a generalized disease; others have reasoned that disordered absorption is the basic process and that resulting nutritional deficiencies are manifested by changed cellular morphology and activity. Biopsies obtained at sigmoidoscopy may occasionally reveal evidence of Whipple's disease, lymphosarcoma, and other entities which result in malabsorption. Intestinal biopsy by the oral route during life[7] has provided new impetus to the study of the pathologic changes in these disorders. The technique has also raised interesting questions as to whether the lesions are reversible after therapy is instituted. Changes in the small intestine during the course of nontropical sprue consist primarily of villous atrophy.[7] Such an alteration reduces the surface area available for absorption, and might abolish the ability of individual villi to undergo pumping action. Despite clinical improvement during steroid administration, short term follow up studies appear to indicate that the histologic picture does not change; perhaps this represents a basic disorder of the mucosa, independent of clinical manifestations.

X-ray studies are useful for both their positive and negative contributions. Negatively, they help rule out other disorders. Positively, they lend weight to a diagnosis of primary malabsorption by identifying an intestinal "deficiency pattern." The pattern consists of segmentation and flocculation of ingested barium in dilated small bowel loops. Such a finding is apparently not present early in the disease, and tends to disappear with clinical remission. Gardner[4] has summarized the literature on the production of barium flocculation by addition of excess mucous, and its significance in malabsorptive states. The mucous in tropical sprue differs from that normally found[36] and is possibly related to a mucous barrier to absorption.

Biopsy of enlarged lymph nodes is almost always indicated, and may be rewarded with evidence of Whipple's disease or lymphosarcoma. Laparotomy is performed when an abdominal mass is present, or when other signs and symptoms are suggestive of a fundamental disorder which has malabsorption as a manifestation.

## ABSORPTIVE STUDIES DURING THE MALABSORPTION SYNDROME

### Water

Excess fluid occurs in the gut lumen in cases of primary malabsorption;[23] this is probably due to defective absorption.[24] Such patients may show an abnormal Kepler-Powers water test (increased nocturnal excretion),[29,30] most likely from the delayed uptake of water.

### Inorganic Molecules

Sodium absorption is slow[37] in malabsorption and returns toward normal during remission.[24] The uptake of $KI^{131}$ is also depressed.[4] Such errors are due not only to malabsorption but also to gross loss in the bulky and liquid bowel movements. Many electrolytes are passed in the excreta, including potassium and calcium. Patients with steatorrhea develop intracellular potassium depletion[48] if the loss continues over a period of time. Calcium loss appears to be more pronounced in patients with 'nontropical' sprue.

### Iron

Iron absorption is defective in primary malabsorption, when gauged by the increase in the blood level after an oral dose.[38] Uptake of radioiron has been studied,[77] but multiple difficulties with this absorption test make its interpretation uncertain.[63] There may be a decrease in body iron stores, and hypoferremia can complicate the other hematologic abnormalities.

### Copper

Copper uptake is perhaps abnormal in primary malabsorption, as blood copper levels are low in many cases.[39] The normal mean copper level is 116 micrograms/100 ml blood, and patients with sprue have been reported with concentrations below 60. Hypocupremia may be associated with hypoferremia and microcytic hypochromic anemia. This possible deficient copper absorption is in marked contrast to the abnormal uptake seen in Wilson's disease (q.v.).

### Carbohydrates

#### Glucose

Patients with malabsorption may have a 'flat' blood curve after an oral glucose tolerance test. However, 40 per cent of the 'normal' population shows the same finding, and hence the procedure is of doubtful value.[38] Hepatic function, pancreatic activity, and other factors influence the final result. The oral glucose tolerance test has little use in either establishing the diagnosis of malabsorption, or in following the course of the disease. Also, rarely, patients with other manifestations of malabsorption may take up glucose normally.

### D-Xylose

This pentose is not selectively absorbed, is not phosphorylated, and does not normally occur in the blood stream. When introduced parenterally, urinary excretion of d-xylose is proportional to the blood level. Following an oral dose of the pentose, absorption occurs and urinary excretion depends on the blood concentration. A tolerance test is based on the ingestion of 25 grams of d-xylose with subsequent collection of urine. Under such conditions, 6.5 ± 1.2 grams of d-xylose appear in the urine within 5 hours.[40] During malabsorption, the blood level is proportionally lower, and urinary excretion is also reduced. Blood assay for d-xylose after an oral dose may be substituted for urine assay. Diminished intestinal absorption of the pentose occurs in conditions other than the primary malabsorption syndrome; however, d-xylose has found widespread use as a screening agent. The defect resulting in reduced d-xylose absorption is perhaps reversible early in primary malabsorption, if therapy is begun, since test values may improve after a period of time. One difficulty with the test is that d-xylose is partially degraded in man (see chapter on Absorption).

### Sucrose

In distinct contrast to glucose and d-xylose tolerance studies, more sucrose is excreted in the urine of patients with sprue than by normal individuals, following oral ingestion.[4] Such a phenomenon is difficult to explain at present, but can also be observed in a group of infants with mental deficiency and hiatus hernia.[41]

## Protein

Numerous cases of sprue have accompanying hypoproteinemia,[33,34] suggesting an error of amino acid absorption[103] or utilization.[42] Oral glycine is slowly absorbed during late stages of the illness,[42] as assayed by the change in blood levels. However, early in the course of sprue, test values may be normal.[43] The major constituents of amino acids (C, H, O, N) do not possess readily usable radioisotopes which emit gamma rays. Hence, studies of radioactive amino acid absorption have not been widely pursued. $I^{131}$ -proteins can not be used here as their absorption depends upon preliminary degradation to amino acids (which is primarily a pancreatic function). A dietary protein, gluten, has a detrimental effect on the course of 'nontropical' sprue (vide infra), but the mechanism of action is uncertain. Reliable studies of protein loss in the feces during the course of the malabsorption syndrome are not available (see chapter on Protein Loss).

## Vitamins

### Folic Acid

Absorption studies utilizing folic acid[44,45,46] must be interpreted in terms of the degree of tissue saturation with the vitamin. A parenteral 'loading' dose to fulfill tissue needs can then be followed by an oral tolerance test. Sampling of both blood and urine is employed after an oral dose. Decreased absorption of folic acid is shown by patients with sprue. The effect of folic acid deficiency on the gastrointestinal tract has been summarized.[64] Since demonstration of the value of folic acid in 'tropical' sprue,[47] it has remained a primary therapeutic tool. Patients with 'tropical' sprue, if given large doses of folic acid without additional vitamin supplements have been known to develop neurologic disorders which resemble those found in pernicious anemia.[57] Hence, until a diagnosis is firmly established, large doses of folic acid should be avoided; when folic acid is used, supplements of other nutrients may be in order.

### Vitamin $B_{12}$

Clinical evidence exists for a dual mechanism of vitamin $B_{12}$ absorption from the gut.[49] These pathways may correspond to the two recently demonstrated vitamin $B_{12}$ binding systems found in the isolated intestine of the rat;[50] one of the systems can be competitively inhibited by vitamin $B_{12}$ antagonists, while the other can not. Clinical tests for the absorption of vitamin $B_{12}$-cobalt[60] have included estimation of the nonabsorbed vitamin in the stool, the Schilling test (counting urinary radioactivity after administering the labeled vitamin by mouth and non-labeled vitamin parenterally),[65] and estimating activity over the hepatic area after oral administration of the compound.[66] In our experience, the

Schilling test is easy to perform and is the procedure of choice.[63,67] Recent evidence indicates that the ileum is the principal site of vitamin $B_{12}$ absorption in man,[115] and that ileal resection or disease may lead to deficient uptake of the vitamin.

The blood level of vitamin $B_{12}$ is low in many patients with malabsorptive disorders.[56] The low blood concentration may persist despite years of therapy,[4] and there is evidence that absorption of vitamin $B_{12}$-$Co^{60}$ is not enhanced by treatment.[51] Patients with sprue show wide variations in their ability to absorb the vitamin[52] and this has been attributed to a minimal absorptive error and a prolonged depletion with time. Purified intrinsic factor has been reported to increase vitamin $B_{12}$ absorption in some cases of sprue,[53] but there is no clear evidence of a failure of intrinsic factor production in this disease. Vitamin $B_{12}$ is recycled, after being excreted in the bile and possibly through the intestinal wall,[54,55] but these pathways have not been investigated in sprue. On the basis of vitamin $B_{12}$ absorption, Oxenhorn and coworkers[12] proposed the following classification of malabsorption.

1. Primary malabsorption.
   a. Impaired absorption of vitamin $B_{12}$ (80 per cent of cases).
   b. Normal absorption of vitamin $B_{12}$.
2. Secondary malabsorption syndrome (such as due to a 'blind loop' of intestine [104,16]).
   a. Impaired absorption of vitamin $B_{12}$, not improved by antibiotics.
   b. Impaired absorption of vitamin $B_{12}$, improved by antibiotics.

In the 'blind loop' syndrome, vitamin $B_{12}$ is perhaps destroyed by bacterial species residing in the loop.[16,104] Absorption of vitamin $B_{12}$ has been shown to be inhibited by a metal chelating agent, and this is reversed by calcium ions.[105] The suggestion has thus been made that decreased vitamin $B_{12}$ uptake during malabsorption is due to a relative deficiency of calcium, as calcium is bound to fatty acids and lost in the stool.

### Other B Vitamins

The absorption of other members of the vitamin B complex in disease states has received little attention in the literature. Three patients with tropical sprue, under treatment, were reported as having normal absorption of riboflavin, thiamine, and pyridoxine, despite impaired uptake of folic acid.[58]

**Vitamin A**

As pointed out in the chapter on Vitamins, carotene is converted to vitamin A in the gut wall. The blood carotene level may be depressed in cases of primary malabsorption (normal value is 60 to 200 micrograms/100 ml), but the significance of this in terms of disordered absorption or conversion is not clear. Vitamin A is a 'fat soluble' nutrient, although water soluble forms are available. The uptake of this vitamin may be connected with the phenomena of lipolysis, particle dispersion, and other aspects of fat absorption (a subject imperfectly understood at present). Blood levels of vitamin A in sprue are of little value, since most patients will have been on massive vitamin supplements. In one series of individuals with tropical sprue, the blood vitamin A level of fasting 'normal' subjects overlapped that of patients with the disease.[61] Blood levels of the vitamin vary with exercise, emotions, and multiple other factors;[59] hence, most reports on 'fasting' patients must be interpreted with caution, unless full particulars are given as to how the subjects were prepared for the venesection. A number of tests of vitamin A absorption have been reported,[60] but these too must be accepted only with caution. The demonstration that vitamin A may be discharged into the peripheral circulation, from intestinal mucosal cells, only after adequate contractions of the gut have occurred,[1] makes specification of the patient's immediate diet of importance. The vitamin A tolerance test[68] has been relied upon by many clinics in establishing a diagnosis of malabsorption; uncritical use of the procedure, however, adds nothing to other studies.

**Vitamins D, E, K**

Decreased calcium uptake, muscle wasting, and hypoprothrombinemia suggest impaired absorption of these vitamins, respectively, in sprue. Reliable studies do not appear to be available, at the present time, to support or refute this hypothesis.

### Fat

Uptake of almost any substance may be altered in malabsorptive states, but fat appears to be most frequently affected. Indeed, the resulting steatorrhea is one of the common and easily recognized (that is, recognized by appropriate chemical means, not visual inspection) manifestations of malabsorption. Just as not all cases of thyroid underactivity are severe enough to result in myxedema, so not all cases of malabsorption are of such a degree as to bring about gross steatorrhea. It is thus unwise to dismiss a diagnosis of malabsorption simply because visible steatorrhea is not present.

Steatorrhea (excessive fecal fat) can be quantitated by determining the per cent of orally ingested fat excreted in the stool; usually less than five per cent of dietary fat escapes via the excreta. The 'coefficient of absorption'[69] is thus greater than 95 per cent. In terms of 'clearance' from the gut,[70] we can say that 95 per cent of the ingested material is apparently taken from the lumen (the word apparent is important, since much of fecal fat is not the ingested material, but consists of parenteral fats excreted across the gut wall). Preferably, the loss is expressed in grams of fat when the oral intake is known. On a diet containing about 102 grams of fat, according to Wollaeger and coworkers,[71] normal subjects excrete less than 6.7 grams of fat in their stools.

Volwiler[13] has discussed the differential diagnosis of steatorrhea; it can not be too strongly stressed that steatorrhea is frequently due to disorders other than primary malabsorption—other conditions (such as deficiency of pancreatic enzymes) must be considered and ruled out. A number of procedures are available for estimating fat absorption,[72] but not all are applicable clinically. Fractionation of stool and intraluminal contents into their component lipids[73,74,75] has received experimental consideration and may be a useful tool in the future in pinpointing the type of digestive error.

As we have discussed, steatorrhea may only be mild and the clinical guidepost of a greasy and bulky stool will then not be present. Staining stool for fat has been widely employed but is difficult to interpret. Further, the test is useless if the patient has recently ingested mineral oil (as the oil takes the stain). In addition, fecal fat is largely in the form of calcium soaps which do not accept stains for neutral fats (they will stain after acid hydrolysis). Sudan fat stains may thus miss even moderate steatorrhea. Fat balance studies are useful if expressed in terms of grams of fat excreted on a standard diet—however, a good laboratory is required. The use of the fat triolein-$I^{131}$ and the fatty acid oleic acid-$I^{131}$ has provided a convenient method of comparing the absorption of lipids between patients[76] (see chapters on Digestion and Absorption).

## SUMMARY OF ABSORPTION STUDIES IN MALABSORPTION

The most reproducible studies of absorption are the Schilling test (vitamin $B_{12}$-$Co^{60}$), the use of radioactive fats, the d-xylose tolerance test, and the chemical determination of fat in the feces after a standard diet. Of little or no use, as commonly performed, are the oral glucose tolerance test and the vitamin A tolerance procedure, for reasons that were discussed. Staining feces for fat is not quantitative, and generally misses fats that have been converted to soaps. While the absorption of many other substances may be abnormal, reliable testing methods are not yet available.

## NOTES ON THERAPY

While there is little agreement as to diagnostic criteria of the malabsorption syndromes, opinion is slightly more uniform concerning the treatment of these disorders (Table 15). Diseases of the pancreas and biliary tree, which result in impaired uptake, are treated with pancreatic and biliary supplements. The use of agents to decrease transit time, in instances of hypermotility with decreased absorption, would appear logical but has been little discussed.

Although no difference has been demonstrated in the bacterial content of the small bowel of individuals affected by malabsorption, as compared with normal persons,[78] the use of antibiotics has gained a role in therapy. Tropical sprue has been reported to improve following administration of sulfonamides[79] or broad spectrum antibiotics,[80] even though no causative organism (or one interfering with absorption) could be isolated. Antibiotics may benefit the malabsorption seen in the 'blind loop' syndrome,[16] and here the mechanism may be related to destroying bacteria in the loop.

Folic acid has been used in the treatment of tropical sprue, while intrinsic factor-vitamin $B_{12}$ gives relief from the specific absorptive defect in pernicious anemia. There is still no understanding as to why folic acid is effective.

Gluten, a protein found in grains, has been noted to exacerbate certain cases of primary malabsorption (particularly in children[81]). No evidence is at hand that an allergic response is involved, and the mechanism of operation is still uncertain. Gluten-free diets have been employed in treatment, often with improvement in tolerance tests and symptoms (vide infra). A brief introduction to the historical background of this topic is given in a paper by Keever and coworkers.[125]

Adrenal steroids have also been employed in treatment of the malabsorption states.[116] Many of the signs and symptoms suggest adrenal hypofunction. At present, the use of corticoids in malabsorption is still empirical, and carries great risks.[82] In debilitated individuals, the steroids have often been claimed to reverse the course of a rapidly progressing malabsorption syndrome. Close clinical supervision is essential, since the complications of steroid administration are potentially as serious as the malabsorption syndrome itself.

Much of therapy is designed for symptomatic relief. Vitamin supplements, electrolytes as indicated, and reduction in fat intake, have all found a place in the treatment of malabsorption states.[14,5] It should be pointed out, however, that the consistency of stool is largely dependent on fat saponification, and production of insoluble soaps. If a very low fat diet is instituted, the subject can not make soaps without fat.[107]

## SITE OF THE MUCOSAL DEFECT

Since multiple insults can result in malabsorption, we are faced with the problem of deciding whether they produce a common biochemical lesion, or if numerous distinct errors are involved. A definitive answer can not be given at this time, but the evidence favors the latter possibility. That is, malabsorption of one or more substances may result from damage to highly specific pathways while other functions of mucosal cells are little affected. On the other hand, gross insults such as anoxia and cell toxins doubtlessly result in derangement of a multitude of activities. It should also be pointed out that malabsorption leads to nutritional deficiencies and further biochemical abnormalities. For example, the finding of increased excretion of p-hydroxyphenylacetic acid (a metabolite of phenylalanine) by patients acutely ill with nontropical sprue[108] may perhaps be related to the attendant inanition and depression of enzyme systems.

A number of observations on altered tryptophan metabolism in malabsorption stand as isolated findings which may one day fit into a coherent picture. Urinary excretion of 5-hydroxyindoleacetic acid, a tryptophan metabolite via serotonin, has been shown to be elevated in patients with nontropical sprue.[110,111] Urinary excretion of skatole derivatives, derived from tryptophan, is elevated in cases of malabsorption.[112,113] These substances, possibly formed by bacterial action, are cellular toxins; whether they are of importance in the genesis of malabsorption is unknown. There may also be considerable excretion of indoleacetic acid.[109]

The deleterious effect of a wheat protein (gluten) on certain cases of malabsorption is exhibited by both the intact protein and its peptides.[81] Complete hydrolysis of the peptides to their component amino acids is followed by loss of the toxic affect. This suggests that the offending agent is a combination of amino acids (a peptide). Even normal animals show decreased absorptive abilities after the feeding of gluten.[83] In addition, sodium-L-glutamate has been shown to decrease absorption of glucose from the surviving guinea pig intestine.[84] Since gluten contains glutamic acid and glutamine, this would appear to be a logical starting point for an investigation of the toxic factor. Following feeding of gluten, blood glutamine rises to a higher level in patients with malabsorption than in normal individuals.[114] This suggests that:

a. normal individuals convert part of the absorbed glutamine to glutamic acid (such an enzyme system is known to exist in the gut)[85], whereas patients with malabsorption do not, or

b. patients with the malabsorptive disorder produce glutamine from its precursors to a greater extent than normal persons, or

c. there is a defect in glutamine utilization during malabsorption, which may or may not be related to folic acid metabolism.[126]

During experimental small bowel anoxia in dogs, the concentration of a transaminating enzyme can be shown to increase in the blood stream.[87] In man, small bowel anoxia may be followed by malabsorption.[18,19] Whether damage to the transaminating system is among the factors responsible for the decreased uptake is unknown. However, adrenal steroids which often exert a beneficial effect on malabsorption, have been shown to enhance transaminase activity.[86]

Adrenal steroids have also been shown to enhance phosphatase activity,[88] including that within the gut wall.[89,90] The possibility is thus raised that the beneficial effect of the steroids in malabsorptive diseases is related to an effect on phosphorylating systems. This is of potential importance since phosphorylation has been implicated in the absorption of many compounds from the gut lumen. In addition, following a fat meal, there is an increase in the blood level of alkaline phosphatase.[91] The rise in the blood concentration of the enzyme has been attributed to a translocation of intestinal alkaline phosphatase via lymphatic vessels. Such an observation deserves further study, especially since an antibody to dog intestinal phosphatase is available.[92]

Patients with sprue are able to normally carry out conversion of the amino acid glycine to serine.[42] However, serine utilization is depressed. This may be of importance as one of the pathways of serine metabolism is as follows.

glycine ⟶ serine ⟶ ethanolamine ⟶ phosphoethanolamine.

A number of children with malabsorption have been noted to excrete phosphoethanolamine in their urine[93] (a defect which is not specific for this disease [94]). Phosphoethanolamine occurs in the small intestine of pigs and rabbits,[95] and can act as a substrate for alkaline phosphatase.[96] The possibility is raised that the compound is somehow involved in an error of phosphorylation.

At the present time then, there are a number of interesting biochemical leads, but no specific pinpointing of the absorptive errors.

## APPENDIX A

### Whipple's Disease

Whipple's description[97] of a disorder characterized by pigmentation, emaciation, polyarthritis, and diarrhea, introduced an entity whose pathogenesis still escapes definition. The finding of a doughy mass in the abdomen (due to knotted, inflamed small intestine) often brings the patient to operation. By means of surgical intervention or the use of a biopsy tube, a section of intestinal mucosa can be obtained for microscopic examination. The wall of the small bowel is thickened, with the serosa speckled by small white flecks. Dilated, milky-white lymphatics course in the mesentery to masses of enlarged lymph nodes. The diagnosis of Whipple's disease is practically secure with these findings, and is confirmed by the finding, in the tunica propria, of macrophages which contain material readily accepting the PAS (periodic-acid-Schiff) stain.[99] Whipple in his initial report of the disease, described the presence of excess lipid in the small intestine as well as engorged macrophages. Due to the lack of a more specific name, the disease was called "intestinal lipodystrophy." Such a designation is untenable, as Black-Schaffer [100] described the lack of lipids in the macrophages but was able to show that they contain PAS-positive substances. Metachromatic stains are also taken by the intracellular material, which has led some to postulate an origin from the breakdown of the 'ground substance' of the intestine (and hence that Whipple's disorder is a 'collagen disease'). Characteristic macrophages can occur in organs other than the intestine during the disease, suggesting a systemic disorder.[107] The possibility is of course present that the abnormal macrophages are not engulfiing the PAS-positive material, but are manufacturing it. In keeping with the 'collagen disease' concept, total serum polysaccharides and mucoprotein polysaccharides are elevated in Whipple's disease.[101]

Mesenteric lymph nodes are filled with mononuclear cells and fats. This mesenteric lymph node involvement has suggested to some that Whipple's disease is primarily one of the intestinal lymphatics (with mechanical interference to absorption). Too few cases of Whipple's disease have been collected in any one series to try to relate the disorder to a genetic disturbance.[11] Adrenal steroids have offered some relief from the disease that is otherwise almost uniformly rapidly fatal.

## APPENDIX B

### Intestinal Lymphatics

Egress of materials from the intestine takes place either through the blood stream or lymphatic channels. Up to this point, we have paid scant attention to the latter pathway because little is known of its precise functioning. This is, of course, not fully justified. Intestinal lymphatics have been described anatomically,[117] their congenital defects are of clinical importance,[118,119,120] and experimental techniques are available for the collection of lymph fluid as it leaves the intestine.[121]

According to the partition theory of lipid absorption (see chapter on Absorption), part of ingested lipids are absorbed into intestinal lymphatics and part (the short chain fatty acids) enter the portal circuit. Whether this partition changes during malabsorption is unknown. It has been recognized, however, that blockage of intestinal lymphatics (by lymphomas[20] or Whipple's disease) can result in diminished uptake from the gut lumen. Perhaps it is true that malabsorption from the intestine, at least for substances absorbed into the lymphatics, can be produced by blocking the outflow of lymph from the area. The dramatic extent to which certain materials enter intestinal lymphatics, and not the portal circuit, is shown by studies on the absorption of clostridial toxin from the gut.[122] When the toxic substance is placed in the intestinal lumen, it appears entirely in the thoracic lymph; cannulation of the lymphatic outflow protects the animal from the otherwise noxious effects of the clostridial product.

Intestinal lymph is likely influenced by the presence of electrolytes.[123] It serves as a means of conveying at least one enzyme (alkaline phosphatase) from the gut to the general circulation.[124] Lymph passes through lacteals of the intestinal villi, where it may function in the absorption of nutrients and perhaps in the exchange of cells with the villi.[62] Study of these reactions is a largely unexplored area.

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## 24

# SUPRA-ABSORPTION: HEMOCHROMATOSIS AND WILSON'S DISEASE

In the previous chapter it was pointed out that the term malabsorption referred, in the broadest interpretation, to either pathologically diminished or augmented uptake from the alimentary lumen. While it is easy to conceive of diminished absorptive capacities due to mucosal disorders, it is not as simple a matter to visualize greater than normal absorption. The concept is more readily grasped if we first realize that the intestine does not possess unlimited absorptive powers. For instance, absorption of glucose does not exceed 0.18 gm/50 cm gut/kilo per hour.[42] These quantities, however, are large compared with the amounts usually ingested; hence little limit to uptake is normally encountered. This is true of many substances in the diet—absorptive abilities of the gut are adequate to insure nearly complete uptake of metabolites taken orally. Absorption in this range of intake is dependent upon the amount presented to the gut, and not on body need for the material. Certain electrolytes, however, such as calcium and magnesium, form a second class of materials which are only fractionally absorbed (only a fraction of the oral dose enters the body) . The limiting factors here may be low solubility, the presence of chelating agents within the lumen, and perhaps mucosal discriminatory mechanisms. In any event, there are no presently recognized disorders which *selectively* affect uptake of these fractionally absorbed materials.

There is also, it appears, a third class of substances which are absorbed from the lumen. These are materials whose entry into alimentary lumen cells is carefully controlled by intracellular factors, and whose uptake depends upon body need. There is evidence that iron, and perhaps copper in addition, is handled this way. In such instances, limits to absorption are detectable rather easily, since the body is (teleologically) working to prevent the entrance of excess amounts. It is of interest that the uptake of iron and copper, normally under rigid control, may be involved in pathologic states manifested by an absorption which is greater than normal (supra-absorption).

## WILSON'S DISEASE

This disorder is a recessively inherited condition. In the fully developed form, opaque rings are found in the cornea (Kayser-Fleischer), there is liver disease, and neurologic manifestations are prominent. The descriptive name of hepatolenticular degeneration refers to involvement of the liver and the lenticular nuclei of the brain. This entity remained a clinical curiosity until it was demonstrated that excess copper could be found in the brain, liver, and corneal rings of affected individuals. Deposition of excess copper means either: 1) abnormally elevated copper absorption, or 2) depressed copper excretion. There is evidence that both factors are operative in Wilson's disease.

An important finding in understanding Wilson's disease was that the blood level of ceruloplasmin, the plasma protein that transports copper, was lower in affected individuals than in the general population. Excessive uptake of copper from the alimentary lumen has been noted in Wilson's disease.[35,36] This is likely a true increase in absorption, and not a decrease in copper secretion into the alimentary lumen, as the copper content of bile is normal during the illness.[39] Why more copper is taken from the gut lumen in Wilson's disease is unknown. It has been proposed that absorption of copper from the gut depends upon a concentration gradient.[37] Hence increasing the concentration gradient (as would occur if no, or very little, copper were present due to an absence of ceruloplasmin) increases absorption. It will be recalled that according to Fick's law,

$$\frac{dm}{dt} = D\ A\ \frac{dc}{dx}$$

the amount of material diffusing per unit time (dm/dt) is equal to the diffusion constant (D) times the effective surface area (A) times the concentration gradient (dc/dx, where c is concentration and x is distance) . Hence a larger change in concentration between lumen and blood stream could conceivably be accompanied by elevated absorption. The same general type of reasoning is applicable even if copper uptake was not entirely a passive process. The difficulty with such an argument however is that administration of ceruloplasmin to affected individuals does not correct the absorptive defect.[36]

With little ceruloplasmin available for copper binding, the excess copper appears to be deposited in the cornea, liver, and brain of patients with Wilson's disease. Pathogenesis of the disorder may actually be far more complex than this, for there is accompanying renal damage with amino aciduria. Therapy has consisted of attempts to mobilize copper from body stores, and attempts to reduce copper intake. Copper is present in many foods and is particularly abundant in shellfish. In attempts to render some of the dietary copper unsuitable for absorption from the gut, oral use of potassium sulfide and various resins has been proposed.[38]

## IRON ABSORPTION AND HEMOCHROMATOSIS

Heavy metals are absorbed to a varying extent from the gastrointestinal tract. The logical assumption was that iron followed a similar pattern, with excretion maintaining the body in iron balance. In 1937, this view was brought into question by McCance and Widdowson.[11] These investigators put forth the hypothesis that iron absorption was controlled, in some manner, by the intestinal wall. (This should not be interpreted as meaning that there is no absorption and secretion by individuals segments,[12] or no exchange diffusion, but rather that total absorption is slight and secretion of iron by the alimentary tract is also minimal.) The evidence in support of the suggestion of McCance and Widdowson is several fold.

1. Following parenteral iron administration, there is almost no excretion of the material in stool, urine, bile, perspiration or sloughed cells.[11] Hence, once iron enters the animal body, it has difficulty in getting out. It has been observed that repeated blood transfusions result in the accumulation of iron (hemosiderin) in the body (each 100 ml of blood contains approximately 50 mg of iron; total iron in the body is 4 to 5 grams) . Further studies, utiliz-

ing radioactive iron ($Fe^{59}$), have amply confirmed the observation that body iron does not readily escape.

2. By the use of radioiron, it can be demonstrated that relatively little of an oral dose is normally absorbed. In animals or men made anemic by blood loss, the uptake of oral $Fe^{59}$ is increased many times.[13] In other words, there appears to be a block to iron uptake, which is abolished during anemia. This effect usually does not take place until six or seven days after a massive hemorrhage,[10] and oxygen deficit has been postulated as being the mechanism involved. Other work indicates that, even in the presence of adequate iron stores, a low hemoglobin level is followed by increased iron uptake.[14,15]

3. Iron in the mucosal cell depends on dietary iron. Only traces of iron are normally present in the intestinal mucosa of the guinea pig. Upon Fe intake, mucosal stores rise in 2 to 3 hours, and stay elevated for 2 to 5 days.[16] The form of iron present in the alimentary mucosa is known as ferritin. This is a brown, iron-protein complex, which may be obtained by cadmium sulfate precipitation.[17] The compound has a molecular weight of about 460,000. Crystalline ferritin contains up to 23 per cent iron (dry weight), but there may be other noncrystalline forms of the substance. Upon removal of iron from the ferritin molecule, a colorless protein (termed apoferritin) is formed, which is also crystallizable upon addition of cadmium sulfate.[17] Since the protein, apoferritin, crystallizes whether iron is present in its molecule or not, it is surprising that it is not found in the gut wall. A plausable explanation is that apoferritin is continually being formed and broken down, but that breakdown is prevented (or synthesis encouraged), in the presence of iron. Hence iron feeding leads to an accumulation of ferritin (apoferritin + iron $\longrightarrow$ ferritin). Once ferrous iron is absorbed, it is converted in the mucosal cell to the ferric state, probably through the action of an enzyme system.[17] The details of iron absorption and metabolism within the mucosal cell have been schematized.[18]

Ferritin itself may have three functions.

1. Normal iron storage (especially in the intestine, spleen, liver, bone marrow, and to a lesser extent in other organs).

2. Regulation of iron absorption from the intestine. Ferritin is also found in the placenta,[17] and the suggestion has been put forth that its presence prevents the passage of excess iron from the mother to fetus.

3. Ferritin inhibits the vascoconstrictive properties of epinephrine, during shock.[19]

Normal iron metabolism may be summarized as follows.

1. The healthy adult shows a 'block' to iron absorption. However, several days after an acute hemorrhage, iron absorption increases several fold.

2. The passage of iron into alimentary mucosal cells is practically 'one way.' That is, no appreciable iron is excreted by the cells of the gastrointestinal tract.

3. Upon ingestion of iron, there is a rapid increase, and then a slow decrease, in mucosal ferritin. The formation of ferritin is apparently dependent upon the synthesis of a protein (apoferritin), and its combination with iron. Apoferritin production seems to be stimulated by the presence of iron in the alimentary tract.

4. Iron is drawn from intestinal mucosal cells as needed, principally via the blood. Iron is transported in the ferric form (probably because of oxygen dissolved in the blood stream),[20] principally in combination with a $\beta_1$-globulin (siderophilin). The average plasma iron level is about 130 micrograms per 100 ml for men, and 110 micrograms for women.[21] The siderophilin molecule is normally not saturated with iron; hence additional quantities of the metal may be bound to the protein if necessary. Tissue storage of iron consists mainly of ferritin, along with a second compound (hemosiderin [22]). The hemosiderin moiety is perhaps a ferric hydroxide polymer which may form when ferritin has become saturated with iron; however, such an assumption is unproven.

But to return to the mucosal cell—by assuming that iron is absorbed from the intestinal lumen only when ferritin within the mucosa has fallen below a certain 'physiological saturation,' we have a possible explanation of the mechanism of normal iron uptake. This is a complex cycle; does it ever 'breakdown'? Mention has been made that in both anemia and pyridoxine deficiency (which may induce anemia in animals), iron absorption can be observed to be augmented. What would be the effect if iron absorption were to be increased, even slightly, for a prolonged period of time? Let us consider an example.

*Normal:* 10 mg ingested x 20 per cent absorbed = 2.0 mg taken up.
*Abnormal:* 10 mg ingested x 25 per cent absorbed = 2.5 mg taken up.

By altering absorption only 5 per cent, an additional 0.5 mg will be absorbed per day. Since iron is not significantly excreted, this extra quantity will stay in the body. At the end of five years, if this rate continued, nearly one extra gram of iron would be present (it will be recalled that total body iron is normally 4 to 5 grams). Such a state of excessive iron accumulation is known to occur, and is termed hemochromatosis.

There are several points that should be mentioned in connection with hemochromatosis.

1. The exact nature of the abnormality is unknown. Apparently, only a minute defect in absorption, as illustrated above, will result in accumulation of the metal. Granick[17] postulated a mucosal error, with an alteration of the cellular oxidation-reduction level (so that there was a disturbance of the normal gradient, which had more $Fe^{++}$ at the luminal side of the cell, and greater $Fe^{+++}$ toward the milieu interieur). This however, is conjectural, and why it should occur is unknown. There is some evidence that hemochromatosis may be an inherited defect.[23,24] The abnormal iron uptake in hemochromatosis is demonstrable even after repeated phlebotomies have removed large amounts of blood.[40]

2. Despite conflicting reports in the past, it is apparent that excess deposition of iron is noxious to tissues, and will cause damage. This can be inferred from the fibrosis and disorganization shown by organs which contain pathologic quantities of the metal. Such a conclusion is also supported by observations that:

A. Iron can denature proteins.

B. At least one enzyme system is inhibited by iron.[25]

C. As a heavy metal, iron may bind sulfhydryl groups, thus exposing tissues to the damaging effects of other agents.

3. The disease, hemochromatosis, can be explained adequately by the presence of excess iron within the body. Thus, the condition is rare in menstruating women, because they lose blood (and iron)

in the menstrual flow. It has also been recognized that hemochromatosis (or a closely related disease complex), can be produced in human beings either by repeatedly administering blood (50 mg Fe per 100 ml), or by having the individual ingest excessive quantities of iron for long periods of time.

The storage form of iron is of importance, since it is at the storage level that the effects of hemochromatosis are manifested. Approaches to the evaluation of body iron stores have been described.[41,18,26,27] Ferritin contains only a limited amount of iron. What happens to iron after this saturation point has been reached is unknown. The suggestion that excess iron may be stored in a second form—hemosiderin, appears to have merit.[17]

Althausen and Sborov[28] have presented some aspects of the clinical picture and prognosis of hemochromatosis. Excess iron is deposited in many tissues (including the heart and endocrine glands), but the most striking affinity is for the liver, pancreas, and skin. This produces the triad of hepatomegaly, diabetes, and bronzed skin ('bronzed diabetes').

The liver is enlarged, with deranged architecture, and altered function. Hemochromatosis is often associated with skin darkening, probably due not so much to the color of iron itself, but to the presence of melanin (which forms when -SH groups are inhibited by the heavy metal; see also the chapter on Pigmentary Disorders). Involvement of the pancreas can result in sufficient destruction of islet cells to produce diabetes mellitus. Suspicion is most often aroused by the finding of hepatomegaly and skin darkening in a patient with diabetes mellitus. Several procedures may be helpful in establishing the diagnosis.

1. Blood iron content is elevated.
2. Skin biopsy may show the presence of excess iron.
3. A biopsy of the gastric mucosa may reveal hemosiderin in the tubular glands, while the parietal cells are practically free of the pigment.[29]
4. Hepatic biopsy can show structural alteration and iron deposits.
5. Absorption of orally administered $Fe^{59}$ can be elevated, but the procedure is frequently unreliable.[33]

The relative value of these procedures has been discussed.[28,29] It must be realized that 80 per cent of those with hemochromatosis will show the characteristic triad of hepatomegaly, skin pigmentation, and diabetes mellitus. The disease should be suspected in anyone giving a family history of a similar illness, but a negative family history certainly does not rule out having the malady.

Therapy follows essentially three lines.

1. Limiting iron intake.
2. Removing iron from the body.
3. Treating the liver and pancreatic afflictions symptomatically.

Theoretically, iron intake can be reduced by: (a) prescribing a diet low in the metal, (b) giving antacids to raise the gastric pH (converting ferrous iron to the ferric form, which is less soluble), (c) avoiding ascorbic acid and sulfhydryl groups as much as possible (they aid the conversion $Fe^{+++} \longrightarrow Fe^{++}$), and (d) the feeding of phytic acid to bind iron in the gut.

Removal of iron, once it is in the body, is a difficult task. Repeated phlebotomies have been employed, on the assumption that iron in storage depots is in equilibrium with blood iron. Usually by the time the disease is recognized, there is severe tissue damage, and iron removal at such a time may not cause reversion of the lesions. Chelating agents potentially can find use in iron binding in vivo. Since one of the pathologic effects of iron is to bind sulfhydryl groups, there is a rationale for using agents containing the -SH moiety (parenterally, not orally).

Symptomatic treatment is directed toward a diet that will not place too great a demand on the damaged liver, and the administration of vitamin K when indicated. The diabetes must be managed by conventional regimes of dietary control and insulin.

As compact and logical as the 'ferritin block' theory once looked, there is evidence that this is not the complete story of the regulation of iron absorption from the gut. For instance, it has been shown that at the time of maximum ferritin accumulation in the duodenum, iron absorption may still occur.[43] Hence a total mucosal block was not present; at least one other (albeit as yet undefined) pathway was operative. The significance of this observation has still to be evaluated.

There are a few additional aspects of iron absorption which merit attention. Attention has been paid to the form of iron available for absorption,[1] and a two compartment classification has been employed. Iron in the first form reacts with the reagent $\alpha$, $\alpha$-dipyridyl (ionic iron), whereas iron in tight organic combination does not react with the reagent. The most facile assumption would be that the $\alpha$, $\alpha$-dipyridyl reacting fraction (referred to as 'available iron') was entirely absorbed while the iron-organic complexes were not suitable for uptake. In vivo, an iron salt which was fully reactive with $\alpha$, $\alpha$-dipyridyl might only be 20 per cent absorbed. Further, part of the iron that is 'nonavailable' to the reagent, may be physiologically available to the body.[2,3]

Certain factors which alter iron absorption have been identified.

1. The divalent (ferrous) form is more readily taken up than the trivalent (ferric) form.[4] This is perhaps partly due to the greater solubility of ferrous complexes; ferric hydroxide for example, tends to polymerize, forming an insoluble material. Substances which aid the reduction of $Fe^{+++}$ to $Fe^{++}$, such as ascorbic acid and sulfhydryl groups,[5] potentially can improve absorption by making the ferrous form more readily available. Iron may be absorbed throughout the alimentary tract, but the major portion is taken up from the duodenum.[10] Passing into the duodenal cap, iron is largely in the ferrous form after having been exposed to the acid gastric contents. Beyond this point, the pH rapidly increases, due to the influx of alkaline pancreatic juices. However, even when supplied in the right valence form, iron uptake is influenced by various factors (for example, copper deficiency diminishes iron uptake,[8] while pyridoxine deficiency increases iron absorption in experimental animals,[9] perhaps by producing an anemia).

2. Ferric ions are reduced to the ferrous state in acid media. Hydrocholric acid from the stomach thus exerts a beneficial effect on iron uptake,[6] by maintaining the pH at a lower value than would be present in its absence. This factor, however, is contributory, and not critical, since individuals with achlorhydria may not have an anemia of iron deficiency.

3. Absorption of iron is dose dependent, in two ways. First, at low doses, absorption is high (if 15 mg were fed, and 20 per cent of

this absorbed, 3 mg would be taken up). At high dose levels, absorptive efficiency is low (a typical value might be 5 per cent of a 140 mg dose, or a 7 mg uptake). Hence, more iron is absorbed at the higher dose schedule, even though net efficiency is decreased. Clinically, it is therefore logical to administer as large a dose of iron as can be tolerated, until blood and tissue deficits are replaced (the danger of prolonged over dosage has been pointed out). Interestingly, rats on a deficient diet had a greater iron uptake than normal, and also showed iron deposits in the colon but not the duodenum.[34]

4. Iron reacts with a number of substances within the gastrointestinal lumen. Ferric ions combine with phosphate to form insoluble complexes. Appreciable quantities of phosphate in the diet will result in a diminution of iron absorption. The converse of this, increased iron absorption in the absence of phosphate, may perhaps occur.[7] Small amounts of calcium favor iron absorption by combining with phosphate ions and by binding dietary phytic acid, which might otherwise have combined with iron.There are, in addition, other organic substances which complex iron. Hence, oral iron should be given during periods when the upper alimentary tract is relatively free of food. It can be shown that radioiron administered with a meal is less readily absorbed than if given alone.[30] When iron is taken on an empty stomach, some individuals report adverse reactions; at least part of this is psychological in origin.[32] Complexes of iron with various organic materials have been tried in order to avoid gastric irritation. The true value of such preparations is unknown. There are still other factors which influence the absorption of iron. For example, children have a greater absorption per unit dose than adults.[31]

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## 25

# EFFECTS OF IRRADIATION

## EFFECTS OF IRRADIATION ON THE GASTROINTESTINAL TRACT

THE USE OF radioisotopes in diagnostic procedures is known to most physicians, but the other side of the coin, the pathologic effects of irradiation, are usually neglected. Perhaps it would be fairer to say 'usually not considered,' as radiation illness is seldom mentioned in the differential diagnosis of severe intestinal complaints. This certainly requires correction since radiation sickness can be observed in patients undergoing irradiation for malignancies, and in individuals exposed to large doses of ionizing radiations from radioisotopes or fission processes. Often the course of radiation sickness is dominated by gastrointestinal signs and symptoms. This chapter will consider some of the effects of radiation on the alimentary canal and will deal with radiation sickness only as it is mirrored in these effects.

## RADIATION SPECIFICALLY DIRECTED AGAINST THE GASTROINTESTINAL CANAL

Irradiation of the stomach has been proposed as a means of reducing hydrochloric acid production in patients with peptic ulcer. The potential danger of radiation necrosis and perforation, as well as uncertain late effects as regards malignant changes, have placed the procedure in an uncertain status (Clinical aspects of gastric irradiation have been summarized [1,53]). For these same reasons, the treatment of alimentary tumors by irradiation has not been widely advocated. Irradiation has also been proposed in the therapeutic regimen against regional enteritis. At present, there is no sound basis for such a proposal and intestinal irradiation is best avoided.

The lower regions of the colon stand in proximity to the urinary bladder, the prostate gland in men, and the cervix in women. Application of large doses of irradiation to these areas, occasionally used in treating neoplasms, may subject the bowel to considerable radiation. The implantation of radioactive pellets, or the use of a directed beam of x-rays or gamma rays, on surrounding tissues, can excessively expose the large intestine to damage unless scrupulous attention is paid to irradiation dosage schedules. The dangers of intestinal wall breakdown, fecal fistula, infection and scarring[54] makes extreme care mandatory. Small doses of irradiation are also delivered to the gut by diagnostic and therapeutic exposures to x-rays. Tables may be used to calculate the dose as a function of the initial beam and depth within the body.[47]

## EFFECTS OF WHOLE BODY IRRADIATION ON THE ALIMENTARY TRACT

Following exposure to radiation, nausea, vomiting and anorexia may occur. These manifestations of radiation sickness were probably first described by Walsh[2] two years after the introduction of Roentgen's apparatus. Only recently has the relationship of the gastrointestinal tract to the symptoms been elucidated. Wang and his coworkers[3] demonstrated that vomiting which followed x-irradiation depended upon a central chemoreceptive trigger zone in the brain (immediate vomiting) and visceral afferent impulses (vomiting later in the illness). Chlorpromazine affects the chemoreceptor trigger zone to render it less sensitive to otherwise appropriate stimuli,[4] and thus has some use in reducing post-irradiation vomiting. In the rat, part of the early response to whole body irradiation is an alimentary antiperistalsis which regurgitates intestinal contents into the stomach;[48] this may be one of the reasons for post-irradiation vomiting. Anorexia has also been related to the extent of alimentary damage by radiation, and can be used as an approximate clinical guide to prognosis; the mechanism may involve the degree of sloughing of the intestinal mucosa.

Damage to the intestinal mucosa of animals subjected to irradiation was reported as early as 1906.[5] The observation was also made that the duodenum and jejunum appeared more susceptible to injury by x-rays than the stomach or colon.[6] Crypt epithelium, which possesses a high rate of regeneration, is the most sensitive area of the alimentary tract to the detrimental action of radiation,[49] and one of the most sensitive tissues in the body; this is consistent with the observation that crypt cells are the ones most readily damaged by colchicine (which is also an inhibitor of mitosis [7]). Symptomatology (as diarrhea), however, may bear little relationship in time to the gross histologic changes.[8] Lethal doses of irradiation result in damage to the entrie mucosal lining. Crypt cells are fragmented, epithelium is sloughed leaving denuded villi and ulcers, and hemorrhage and edema are prominent. Changes have been reported as early as one hour after irradiation.[9] The physical conditions of irradiation (fractionation of the dose, type of particle and its relative biologic effectiveness,[50] the degree of shielding and so on) as well as biologic factors such as age[10] all influence the extent of damage produced. The claim has even been made that crypt epithelium may acquire radioresistance with properly applied small exposures over a period of time.[11]

Precise biochemical alterations produced by irradiation, which are the cause of the morphologic and functional changes, are uncertain. The following changes are some of the many that have been noted; they should be interpreted in terms of the dose administered, the time after exposure that the analysis is made, and the physical factors of irradiation.

**Physical.**

The dry weight of the intestinal mucosa decreases during the first 48 hours following radiation. The nucleic acid, protein, and ash content of crypt epithelium all decrease following exposure to neutrons or x-rays.[12]

**Synthesis.**

X-irradiation depressed both deoxyribonucleic acid and ribonucleic acid synthesis in the intestine of rats and rabbits, while protein synthesis was less affected.[13] Nucleoprotein of the intestinal wall was degraded by X-rays.[14]

**Enzymes.**

Following neutron irradiation, alkaline phosphatase activity of crypt cells was increased at pH 9, but normal at pH 7.[15] Alteration of enzymes continues after cessation of irradiation; this 'after effect' has been noted with trypsin[16] and pepsin.[17] The increased motility and tonus of the intestine following irradiation in vivo and in vitro,[18] is probably the result of decreased intestinal cholinesterase activity.[19,20,21] Carboxypeptidase activity increases after irradiation,[22] but gastric peptic action probably decreases.[23]

**Respiration.**

The respiration of rat small intestine was inhibited several hours after 700 roentgens whole body irradiation.[24] Oxygen consumption was normal at 24 hours but again depressed at 72 hours. Apparently there was no effect on anaerobic glycolysis. Following irradiation, glucose oxidation of rat small intestinal mucosa in vitro is increased, due to greater activity of the citric acid cycle.[56]

**Electrolytes.**

An exchange of sodium in place of potassium within intestinal cells occurs after irradiation.[25] Loss of electrolytes during post-irradiation diarrhea may be a major cause of death; relatively more sodium than potassium is lost;[26] part of the electrolyte loss is via the biliary fluid.[52]

**Absorption.**

Studies of intestinal absorption following irradiation are hampered by the facts that gastric emptying is delayed[27] and intestinal motility is altered.[18] Each of the absorptive mechanisms may be affected to a different extent.[28,29] Barring massive doses of irradiation to the intestine, most nutrients may be absorbed at almost normal rates.

**Fat Excretion.**

Fecal fats are increased following irradiation. There is evidence that the lipids arise from intestinal secretion rather than from malabsorption or tissue sloughing.[30,31]

## RADIATION DEATH AND THE ROLE OF THE INTESTINE

Massive abdominal or whole body irradiation leads to death in three to five days. In mice, this occurs only if a large part of the intestine is irradiated,[32] and has lead to the concept of an "intestinal radiation death."[33] The mortality is probably due to a number of factors.

1. Damage to intestinal crpyt epithelium precedes the maximal mortality of irradiated mice,[11] and most likely permits the entry of bacteria into the body. If the bacteremia of intestinal origin is responsive to antibiotics, survival may be prolonged.[34] It has been reported that the bactericidal power of human serum, against Gram-negative bacilli, is not decreased following irradiation.[57]

2. Excessive loss of water and electrolytes, during the diarrheal episodes following intestinal irradiation, substantially contributes to death.[26] Relatively more sodium is lost than potassium and it principally leaves via the feces. The suggestion has been made that species differences in survival, following irradiation, may be partly explained on the basis of the length of the large intestine. An animal with a longer colon would have more of an opportunity to reabsorb fluids and electrolytes, thus diminishing loss of these constituents in the stool.[35]

3. Following irradiation, the circulating blood volume decreases. The decrease is less severe if the ileum is shielded,[36] suggesting that the major fraction of blood volume lost passes into the intestinal lumen. Blood volume replacement is necessary to correct for this loss and also to obviate any induced bleeding tendency.

The correlation of gastrointestinal symptoms to radiation illness at various levels of exposure, and modification of the illness have been discussed by Conrad.[35,37]

## ABSORPTION AND SECRETION OF RADIOACTIVE MATERIALS BY THE GUT

Absorption of radioactive materials from the gut forms the starting point for most clinical tests which employ isotopes (see, for instance, the diagram by Spencer *et al.*[38] on the passage of radioiodine across the mucosa). Isotopes of an element are usually handled in identical fashion.* That is, $I^{131}$ (radioactive) is absorbed and metabolized in approximately the same manner as $I^{127}$ (stable); it may thus be used as a convenient 'tag' in following iodine distribution. Secretion of materials across the gut wall, from body to lumen, can also be studied by the use of radioisotopes; recycling of numerous substances is thus demonstrable (iodine, vitamin $B_{12}$, water, bile constituents, cholesterol).

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*The notable exceptions are the isotopes of hydrogen. Deuterium ($H^2$) is twice as heavy as normal hydrogen ($H^1$), and this discrepancy is expressed in some biologic systems by a discrimination against the heavier isotope. As the total weight of the element increases, discrimination against isotopes becomes less significant. Thus $C^{14}$ is metabolized only slightly slower than $C^{12}$, and by the time still heavier nuclides are considered, there is little or no discrimination.

Most isotopes used in clinical diagnostic procedures have a half-life under 100 days. In addition, they are often subject to removal by one or more excretory pathways. Accidental ingestion of excessive amounts of such isotopes ($I^{131}$, $Cr^{51}$, $Fe^{59}$, $As^{74}$, $Cu^{64}$, $K^{42}$, $Na^{24}$, $Co^{60}$, $P^{32}$) hence is thus not invariably associated with great general body irradiation. Certain radioisotopes, like their stable counterparts, are poorly absorbed from the gut and transit the alimentary canal with deliverance of radiation only to the gut wall and nearby structures. Long lived radioisotopes of many of the common elements also occur, but their use in human subjects is rarely justified, since there is the danger of prolonged irradiation of the host.

Elements above atomic number 82 are unstable and exist only in radioactive forms. Isotopes of these heaviest elements are particularly dangerous to the body for three reasons.

1. They are 'foreign' to the organism and no adequate provision is provided for their excretion (they are said to have a long 'biological half-life').

2. Certain of them (as radium, strontium) are "bone seekers." That is, they lodge in bone very much as calcium does. From this site, they are not readily mobilized. Further, they may accumulate in discrete areas forming 'hot spots.'

3. Many of the isotopes of the very heavy elements are beta ray emitters and in addition have a long half-life. When once encamped in tissues, they can continually irradiate nearby areas.

It is apparent that the best defense against long lived isotopes of the heavy elements is to avoid them completely. When they are ingested, depending on such factors as particle size and chemical form, they may be principally passed in the feces and partially excreted in the urine if absorption takes place. After a few days, however, excretion falls off and the isotope remaining in the body is in residence almost permanently. One approach to the problem is to chemically bind the radioisotope in the gut lumen so that it will not be absorbed. The use of various chelating agents (ethylenediamine tetracetic acid, citric acid) has been proposed in attempts to form nonabsorbable complexes with the isotope in the lumen;[39] precipitating agents (magnesium sulfate, oxalic acid) and adsorbing agents have also been mentioned for use, since they may change the chemical characteristics of the isotope within the lumen to a form less soluble, and hence less readily absorbed.[55] These methods may be further developed in the future, but at present their use is uncertain and not wholly effective. Natural mechanisms may also be working to decrease the absorption of heavy nuclides. Thus, plutonium is largely precipitated as an insoluble radiocolloid at the alkaline pH of the gut; even irradiation of the intestine does not increase plutonium absorption.[40] Plutonium absorption is much greater in newborn animals (rat) than in adults,[51] although the responsible mechanism has not been elucidated. Most of the long lived isotopes taken by mouth are principally passed in the feces; methods for determining their presence in the excreta have been described (such as the techniques for detecting radium [46]).

Radioactive materials in the alimentary contents can also arise from the respiratory tree. Radioactive particles from inhaled air may be trapped in the bronchial mucous, coughed up, swallowed, and passed via the digestive tract.

The amount of radiation delivered to the gut by an ingested radionuclide depends upon the length of time it resided in the lumen (or the gut wall), as well as the type of emissions characterizing the isotope. The classic paper of Marinelli, Quimby and Hine,[42] although slightly modified over the years, may be consulted for the general method of calculating irradiation dosages administered by radioactive isotopes. Radioactive phosphorus ($P^{32}$) and radioactive iodine ($I^{131}$) are sometimes administered, in therapeutic amounts, by the oral route. Considerable irradiation of the gut results and gastrointestinal symptoms are common. Pike[45] states that 25 per cent of ingested $P^{32}$ appears in the stool, and hence presumably the entire length of the digestive canal is subjected to irradiation.

The quantity of a radioactive material in the gut wall may be estimated by counting tissue samples in suitable detection devices, or by isolating the radioisotope from the tissue and then counting. Using the latter technique, Lacassagne[41] studied polonium distribution in rabbits and noted small amounts to be present in the intestine. If the ingested isotope was present in a stable 'carrier,' standard analytic procedures or the technique of neutron activation analysis [43] may be used to indicate the amount of carrier.

One of the products of nuclear fission is radiostrontium ($Sr^{90}$). This nuclide is a beta-emitting bone seeker of long half-life (about 27 years). Interest in the isotope centers on the facts that it induces tumors in animals (perhaps also in man), and travels with calcium in metabolic paths. Since strontium and calcium are so closely linked in most biologic systems, it is usual to speak of the strontium/calcium ratio. If a pathway handled strontium and calcium equally well, the ratio Sr/Ca would be 1.00; if strontium were preferentially handled at the expense of calcium, the ratio would be greater than 1.00. Comar, Russell and Wasserman[44] defined an intestinal absorption discrimination factor (DF) as:

$$\mathrm{DF} = \frac{100 - \%\ \text{ingested Sr in feces}}{100 - \%\ \text{ingested Ca in feces}}$$

Two patients on a nonmilk diet had a DF of 0.46, indicating that the gut preferentially absorbed calcium. One patient on a diet containing milk had a DF value of 0.78. This result is not easy to interpret, since milk contains a number of agents which may have interfered with absorption.

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## 26

# PIGMENTARY DISORDERS

There are several clinical entities characterized by involvement of both the alimentary tract and the skin pigmentary mechanism (Table 16). Interest in the subject is of more than passing concern, for proper recognition of the skin changes can focus attention on a gastrointestinal problem that might otherwise have escaped early detection.

From Table 16 it can be seen that no single factor is sufficient to explain all of the observed symptomatology, or to tie all of the syndromes together. The central manifestation in each of the disorders is an aberration of melanin pigmentation, and hence a review of melanin production may serve to shed some light on the problem.

The principal brown pigment of human skin, melanin, is produced by the action of a copper containing enzyme (tyrosinase) on the amino acid tyrosine.[10] The enzyme is located in the cytoplasm of discrete cells (melanocytes) which, after their neuroectodermal origin, migrate to the hair roots, skin, eyes and leptomeninges. Techniques for delineating such cells by their cytology, staining reactions, and uptake of radioactive tyrosine, have been summarized.[11] Tyrosine for body needs comes either from the amino acid in the diet, or from conversion of phenylalanine to tyrosine in the liver.[19]

The triggering action that starts pigment production is usually sun light. By altering the temperature, acidity, and sulfhydryl content of melanocytes, actinic stimulation frees tyrosinase from its inhibitors (principally sulfhydryl groups). The darkening of melanocytes is also under control of a pituitary melanocyte stimulating hormone (MSH). It is in connection with MSH that the

TABLE 16

PRINCIPAL SYNDROMES INVOLVING THE GASTROINTESTINAL CANAL AND THE SKIN PIGMENTARY SYSTEM

| | |
|---|---|
| 1. *Addison's Disease* | Gastrointestinal complaints may be annoying (nausea, vomiting). Adrenalectomy is followed by decreased intestinal ability to absorb Na and K, and to alter the intraluminal osmotic pressure.[1,2] Diffuse bronizng of the skin may occur, perhaps due to increased outpouring of melanocyte stimulating hormone (MSH). |
| 2. *Hemochromatosis* | Basic defect in the disease is an elevated absorption of iron (see chapter on Supraabsorption). Deposition of excess iron leads to tissue damage. Increased pigmentation is perhaps due to Fe binding of sulfhydryl groups, and the escape of melanocytes from normal sulfhydryl inhibition. |
| 3. *Pellagra* | Clinical manifestations of pellagra include gastrointestinal disorders and dermatitis (with hyperpigmentation). Pellagra is the result of prolonged niacin deficiency. Niacin deficiency may decrease—SH groups in melanocytes.[3] |
| 4. *Peutz-Jeghers Syndrome* | An inherited defect manifested by intestinal polyps, and pigmentation of oral mucosa (and often of other areas).[4,5] A number of variant forms have been reported.[6,7,8] How a single gene affects both these sites is unknown. |
| 5. *Danbolt-Closs Syndrome* | This is characterized by remissions and exacerbations of alopecia, photophobia, and skin eruptions principally about the mucocutaneous orifices and lower extremeties. In addition, symptoms of celiac disease are present.[9] Etiology is unknown, but diodoquin has been reported of use in therapy. |
| 6. *Leukoderma During Heavy Intestinal Parasitization* | Many cases of leukoderma in India are associated with protozoan, bacterial or amebic infestations of the intestine. It has been hypothesized that due to the infestation, an absence of the precursors of melanin develops (Sahu, K. C.: Indian Practitioner, 8:1047, 1955); a well documented parallel is interference with vitamin $B_{12}$ absorption when Diphyllobothrium latum (fish tape worm) is present in the duodenum. |
| 7. *Malignant Melanoma* | This uncommon tumor is one of the few which has been reported to frequently metastasize to the gut wall. |

first relationship between the gut and melanocytes becomes apparent. Addison's disease, which may be marked by alimentary symptomatology, is accompanied by darkening of the skin. This hyperpigmentation may be due to an increased production of MSH by the pituitary (which produces large quantities of hormones in an attempt to keep the diseased adrenal gland functioning). Melanocyte darkening normally initiated by MSH can be prevented, in vitro at least, by a number of physiologically active compounds, including epinephrine, norepinephrine and serotonin[12] (see chapter on Carcinoid Tumors). The inhibition of pigmentation by epinephrine, norepinephrine, and serotonin, can be prevented by sympathominetic agents (including ergotamine [12]). The sympathomimetic drugs, of course, inhibit intestinal activity.

Epinephrine is synthesized from tyrosine-like precursors and forms another interrelationship between the gut and melanocytes. The inhibiting effect of epinephrine on gut motility and pigment production in vitro has been mentioned. The latter effect may have some in vivo importance, since vitiligo (localized depigmentation of the skin) is associated in a number of instances with hyperfunctioning of the sympathetic nervous system[3] and hence with an outpouring of epinephrine or norepinephrine.

Brown skin pigmentation, presumably melanin, has been observed in certain cases of primary biliary cirrhosis, sprue, and Whipple's disease. Attempts have been made to equate the pigmentation with deficient intestinal absorption, or with abnormal intermediary metabolism. The ubiquitous epinephrine, which inhibits melanogenesis in vitro, also plays a role in the handling of lipids. For example, experiments have demonstrated that epinephrine is important for lipid mobilization in the rat.[13] Whether excess pigmentation would occur if epinephrine were withdrawn to other pathways (such as lipid metabolism during malabsorption) is open to speculation, and little evidence is available. Lipid metabolism and melanocytes share at least one other common factor—copper. Tyrosinase contains copper as an essential part of its molecule,[10] and removal of the metal renders the enzyme ineffective. Enzymes important in fat metabolism may also require this metallic moiety.[14] In sprue, blood copper levels have been reported to be depressed. Another heavy metal, iron, appears to work at a different point in the chain of melanin production. Excess iron is absorbed in hemochromatosis, and its deposition is various organs results in cellular damage. In the skin, this damage is apparently a lowering of the number of sulfhydryl groups; melanocytes escape from -SH control and produce pigment.

Hyperpigmentation may follow chronic malnutrition, and has also been observed in severe niacin deficiency (pellagra). The syndrome of pellagra involves both intestinal malfunction and dermal pigmentation. Exposed areas of the pellagrous individual commonly become dark, following subsidance of the acute dermatitis.[10] The initial skin reaction possibly causes a fall in the content of intracellular sulfhydryl groups, and hence hastens tyrosinase activity. Gastrointestinal signs and symptoms of pellagra (nausea, vomiting, diarrhea, deficiency of hydrochloric acid secretion), as well as the skin manifestations, revert toward normal upon feeding nicotinic acid (or its derivative, nicotinamide). However, not all dietary deficiencies are accompanied by hyperpigmentation. Kring and Williams have reported[15] that in thiamine deficient rats, the breakdown of tyrosine by a liver tyrosine-oxidase system, rather than being hypoactive was more active than normal.

Melanin itself may be essential for the deposition of other pigments in the skin. Observations on patients with vitiligo, who developed discoloration from jaundice or atabrine ingestion, disclosed the yellowness only in non-vitiliginous regions. Chromogens from bile or the antimalarial drug will not stain hypopigmented skin sections.[3,16]

Peutz[4] recorded a syndrome associating familial polyposis of the mucosa of the small intestine, with skin and membrane pigmentation (1921). Twenty-three years after this communication, Jeghers[5] reported another case, and established historical continuity with the initial description. The entity has since been given the eponymic designation Peutz-Jeghers syndrome. A typical patient has melanotic areas on the face, digital skin, and buccal mucosa, in addition to polyps of the small intestine. Deposited as solid dots or finely stippled aggregations, the melanin pattern is found in affected individuals whether fair skinned or swarthy. Clinically, the syndrome is of importance, as it aids in the detection of intestinal polyps, by simple inspection of the face and digits. These skin findings should alert the investigator to study other members of the patient's family, as the disease has a familial incidence. Variations in the distribution of the intestinal polyps have been described,[6,7] and perhaps a spectrum of abnormalities exists.

An apparently non-familial syndrome involving both intestinal function and skin pigmentation has been described by Cronkhite and Canada.[8] The two women patients had generalized gastroin-

## TABLE 17

### INTERRELATIONSHIP OF A NUMBER OF FACTORS WHICH INVOLVE THE INTESTINAL TRACT AND THE SKIN PIGMENTARY MECHANISM

| | *Melanocyte-Gut Interrelation* | *Effect of Deficiency and Excess* |
|---|---|---|
| *Serotonin* | Produced from tryptophan by intestinal argentaffin cells; excessive amounts made in malignant carcinoid tumors. Contracts smooth muscle, and in vitro inhibits melanogenesis. Released from gut, brain and platelets by Rauwolfia serpentia drugs.[18] | Since made endogenously, impossible to remove all serotonin from body. Skin changes in carcinoid syndrome may be due to niacin deficiency (niacin normally produced at least in part, by tryptophan metabolism). |
| *Niacin* | Sufficient amounts cannot be produced by man, and hence must be absorbed from the diet. | Fully developed deficiency, called pellagra, is associated with skin and gastrointestinal disturbances. Darkening of the skin is perhaps related to a decrease in –SH groups in melanocytes. No major untoward effects from overfeeding niacin. |
| *Melanocyte Stimulating Hormone* | Produced by the pituitary, MSH initiates darkening of melanocytes. The action of MSH is inhibited, at least in vitro, by norepinephrine, epinephrine, and serotonin (the latter causes intestinal smooth muscles to contract). Excessive MSH may be produced in some cases of Addison's disease. | A direct action of MSH on the gut is unknown. During pregnancy, the blood MSH level is elevated. Hypopituitarism has low MSH levels, but intestinal symptoms are probably due to other hormone deficiencies. |
| *Iron* | The essential defect in hemochromatosis is an excessive absorption of iron. In the skin, the Fe may combine with –SH groups, allowing pigment production to proceed. | Iron deficiency can be associated with gastrointestinal difficulties (such as Plummer-Vinson syndrome). |
| *Tyrosine* | Small amounts taken up from diet. The remainder is produced from phenylalanine by a liver enzyme. Tyrosine or its products is a constituent of both intestinal cells and melanocytes. | A deficiency of tyrosine absorption has been postulated in cases of lekoderma accompanying intestinal parasitization. In kwashiorkor, there are skin lesions and alimentary disorders, perhaps due to amino acid deficiency. No toxic effects noted from extra tyrosine in diet. |
| *Copper* | Absorbed from the gastrointestinal canal; excess absorption may occur in Wilson's disease. Copper is part of tyrosinase, and part of certain enzymes important in fat metabolism. | Absence from diet causes depigmentation. Low blood levels in Wilson's disease (due to deficient copper-carrying-enzyme) and in some cases of sprue (? malabsorption). Large quantities of copper can cause alimentary and neural sysmptoms. |
| *Epinephrine* | Produced from tyrosine-like precursors, it inhibits contractility of intestinal smooth musculature. Epinephrine is attacked by certain enzymes which also affect tyrosine. | In vitro, epinephrine inhibits skin darkening stimulated by MSH. Perhaps skin coloration following adrenalectomy may be related to this. Some evidence that vitiligo is associated with over activity of the sympathetic nervous system (and hence release of epinephrine or norepinephrine). |

testinal polyposis (stomach, small bowel, colon), as well as diffuse pigmentation of the skin, alopecia, and atrophy of the fingernails (onychotrophia). Cronkhite and Canada suggested that the ectodermal changes were part of a generalized deficiency state, perhaps related to the extensive polyposis and interference with nutrient absorption. The serotonin content of such polyps has not been reported, and it is not known if the tumor destroys the ability of argentaffin cells to manufacture serotonin (it will be recalled that serotonin inhibits melanin formation in vitro, and perhaps decreased serotonin levels permit pigmentation to proceed). However, case reports of malignant carcinoid with excess serotonin secretion have not mentioned depigmentation of the skin. It has been suggested that the pellagra-like skin changes seen in the carcinoid syndrome, as well as the heart lesions, are related to a niacin deficiency caused by the incorporation of the majority of body tryptophan into serotonin[17] (niacin, the pellagra preventative, is a derivative of tryptophan metabolism). The interrelation of the various substances discussed is tabulated in Table 17. There are, of course, multiple other enzymes and nutrilites common to both melanocytes and intestinal cells.

In summary, a number of syndromes are characterized by involvement of both the gastrointestinal tract and the skin pigmentary system. The mechanism behind this inter-relation probably involves several factors. Recognition of the skin changes may lead to early and correct identification of the alimentary difficulties.

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## 27

# CARCINOID SYNDROME

THE INTESTINAL mucosa contains a cell type which, because if its ability to bind silver compounds, has been referred to as 'argentaffin.' Study of these cells by Erspamer and coworkers[1] resulted in the isolation of a compound which was termed enteramine. In 1952, this group identified enteramine as 5-hydroxytryptamine after Page and associates[2] extracted a vasoconstrictive material from blood serum, and demonstrated that chemically it was 5-hydroxytryptamine.

In 1953, Lembeck[5] reported the presence of 5-hydroxytryptamine in a carcinoid (argentaffin) tumor. This observation assumed clinical significance within a short time of its publication, due to a paper by Thorson and coworkers.[6] Thorson's group described the association of malignant carcinoid tumors of the intestine with a number of bizarre clinical signs and symptoms (flushing and peripheral vasomotor changes, diarrhea, asthma, cyanosis, and lesions of the right side of the heart). They suggested that the findings might be related to the release of serotonin from the tumor (the diarrhea resulting from stimulation of intestinal smooth muscles by serotonin, the asthma from bronchial constriction, and cyanosis from spasm of small blood vessels and inadequate oxygenation). Confirmation of their contention can be found in papers reporting that patients with the carcinoid syndrome possess elevated blood levels of serotonin, and an increased urinary outpouring of 5-hydroxyindole acetic acid (5-HIAA), the final serotonin metabolite.[14] In addition, loading of dietary tryptophan in carcinoid patients results in a rise in urinary 5-HIAA[3] (although clinical improvement has not been noted to follow either tryptophan deprivation or the use of serotonin antagonists).

The metabolism of serotonin (5-hydroxytryptamine, vasotonin, enteramine), a tryptophan derivative, is known in general terms (Figure 15). Hydroxylation of tryptophan to 5-hydroxytryptophan possibly occurs in the gut.[4] The hydroxylated tryptophan is then decarboxylated to 5-hydroxytryptamine. The enzyme amine oxidase, plus a DPN-dependent dehydrogenase,[35] is able to serially convert 5-hydroxytryptamine to 5-hydroxyindole acetic acid,[13] the principal urinary excretory product of serotonin. Serotonin and its immediate precursor, 5-hydroxytryptophan, have dramatic effects on the gastrointestinal tract. Administration of 5-hydroxytryptophan is followed by gastric alterations[7,8] (possibly mediated through conversion of the compound to serotonin). Reactions of the intestine to serotonin depend upon the dose[11] and the method of administration.[12] When applied to the mucosa, serotonin

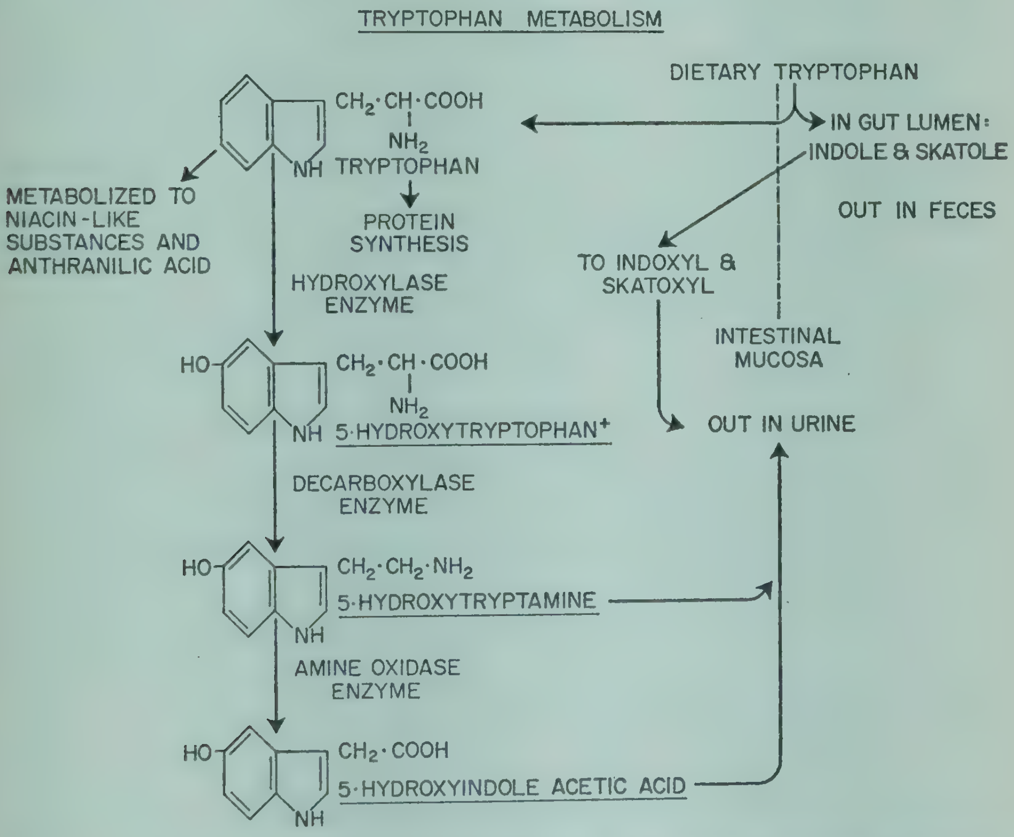
TRYPTOPHAN METABOLISM
DIETARY TRYPTOPHAN
CH2·CH·COOH
NH2
NH
TRYPTOPHAN
IN GUT LUMEN:
INDOLE & SKATOLE
OUT IN FECES
METABOLIZED TO NIACIN-LIKE SUBSTANCES AND ANTHRANILIC ACID
PROTEIN SYNTHESIS
TO INDOXYL & SKATOXYL
HYDROXYLASE ENZYME
INTESTINAL MUCOSA
HO
CH2·CH·COOH
NH2
5-HYDROXYTRYPTOPHAN+
OUT IN URINE
DECARBOXYLASE ENZYME
CH2·CH2·NH2
5-HYDROXYTRYPTAMINE
AMINE OXIDASE ENZYME
CH2·COOH
5-HYDROXYINDOLE ACETIC ACID

Figure 15. Tryptophan metabolism in man. The pathway leading to serotonin production and degradation is emphasized, although normally this is a minor route of tryptophan utilization.

stimulates sensory receptors; when applied to the serosa, serotonin sensitizes smooth muscles to acetylcholine action.[12] The general response is thus an increase in peristalsis. The actions of serotonin are multiple, affecting many organ systems outside of the alimentary tract (see review by Page [9]). For example, serotonin can be found in brain, and certain tranquilizing agents release serotonin from brain and other tissues.[10] Interestingly, rauwolfia alkaloids liberate both norepinephrine and serotonin from the gut.[33]

A typical carcinoid is a slow growing but malignant tumor, most often involving the appendiceal region of the intestine. From its original location, it can metastasize to other areas, particularly the liver. Rarely, other types of tumor mimic the carcinoid syndrome.[15] Clinical descriptions of the carcinoid tumor and its manifestations have appeared[31,32] and may be consulted for additional data. The presence of the carcinoid syndrome can be suspected from the clinical picture, although prior to delineation of the entity, cases were probably passed over as congestive heart failure with unusual secondary manifestations. It seems likely that all carcinoid tumors produce serotonin, and that serotonin in excess amounts results in striking symptomatology. Two procedures are thus available for establishing the diagnosis.

1. Operative removal or biopsy of the tumor.
2. Demonstration of elevated serotonin in the blood, or increased 5-HIAA, the serotonin end product, in urine.

A simple chemical screening test has been devised for demonstrating 5-HIAA in urine.[16] Normal individuals excrete an average of 5 mg of 5-HIAA in a 24 hour urine specimen, whereas patients with the carcinoid syndrome put out many times this amount of the compound (76 to 580 mg in one study [3]). However, no significant 5-HIAA is found in plasma, feces, or cerebrospinal fluid. The blood serotonin level is elevated in patients with the carcinoid syndrome and the compound is almost entirely bound to platelets. Serotonin occurs in large amounts in carcinoid tissue itself,[5] and chemical techniques are available for determining the amount of serotonin in tissues.[17,18]

Production of large amounts of serotonin, from the amino acid tryptophan, appears to be the principal pathway of tryptophan metabolism in patients with carcinoid tumors. Normally, an increased intake of tryptophan exerts little influence of the output of 5-HIAA, suggesting that this pathway is nearly saturated.[19] In contradistinction to this, persons with the carcinoid syndrome demonstrate an excretion of 5-hydroxyindoles that varies with tryptophan intake. In control subjects, about one per cent of ingested tryptophan appears as serotonin. When a carcinoid is present, up to 60 per cent of dietary tryptophan is converted to serotonin.[3] Because of the use of dietary tryptophan to form serotonin by the carcinoid, manifestations of tryptophan deficiency in other metabolic pathways might be expected. This is the possible explanation of the high incidence of pellagra in patients with carcinoid tumors (tryptophan normally is converted to niacin-like substances, see Figure 15). However, the diarrhea attending the carcinoid syndrome may contribute to poor absorption of essential nutrients.

The cardiac abnormalities found in some patients with carcinoid tumors are not as readily explained as the other symptoms. The suggestion has been made that the high concentration of serotonin reaching the right side of the heart (before it is inactivated by passing through the lungs)[23] causes damage to valves and endocardium.[21] An additional possibility is that repeated flushes seen in the course of the disease cause altered hemodynamics and cardiac damage[22] (the flush is likely the result of vascular relaxation which follows a bout of vasoconstriction due to serotonin).

Definitive treatment of carcinoid tumors is surgical removal. Niacin supplements and additional dietary tryptophan have been suggested as temporary measures. Once the diagnosis is established, it would be interesting to follow up reports that systemic disturbances from carcinoid tumors may be precipitated by fatty foods[25] or alcohol.[24] Excretion of 5-HIAA should decrease after removal of a carcinoid tumor;[20] since many of the malignancies have functioning metastases, the urinary 5-HIAA level may fall only slightly after extirpation of the main tumor.

Rauwolfia serpentia causes the liberation of serotonin from tissues,[10] but does not result in significant urinary excretion of 5-HIAA or alterations in uropepsin excretion.[26] (Uropepsin is a urinary enzyme presumably arising from gastric pepsin.) Serotonin excess, seen in the carcinoid syndrome, is associated with a high incidence of peptic ulceration[31] although when administered to dogs, serotonin causes a decrease in gastric acid secretion.[11]

There are three entities, aside from carcinoid tumors, which are associated with urinary excretion of excessive 5-hydroxyindole acetic acid. The first of these is the ingestion of bananas. This fruit contains large amounts of both norepinephrine and serotonin.[27,28] Hence, bananas must be withheld when collecting urine to measure the amount of 5-HIAA present. Second, some patients with the malabsorption syndrome excrete excessive amounts of 5-HIAA.[29,30] The significance of this, in terms of the fundamental biochemical lesion, is not yet apparent. Third, phenylketonurics maintained on a low phenylalanine diet may excrete more 5-HIAA than normal.[34]

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*Note:* There has recently been described the occurrence of neuroblastic tumors with diarrhea and increased urinary excretion of catecholamines. This is likely an entity distinct from the carcinoid syndrome.

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## 28

# INTESTINAL SUBSTITUTES FOR THE BLADDER

Removal of the urinary bladder may be necessitated by tumors or inflammation. With a functioning kidney, life can continue without the bladder, providing a repository is established for urine. Suturing the ureters to the skin, with drainage into a bag, has the disadvantages of skin decimation, bacterial infection, and frequent mal-performance of the collecting sac.

Since the alimentary canal handles waste materials in the form of feces, investigators have considered the possibility that it might accept renal wastes as well. Two basic surgical attacks on the problem evolved.

1. Forming a 'bladder substitute' from a section of the digestive tract, by isolating a pouch of intestine.
2. Draining urine directly into the intact alimentary canal.

A thorough analysis of the problem would apply both techniques to each of the subdivisions of the gastro-intestinal apparatus and compare end results. Such an approach is not available, and certain phases of the study have received more attention than others. Particularly investigated have been the use of segments of stomach,[1] ileum,[2] ileocecum,[3,4] and especially sigmoid.[5] Study has also been made of the use of reversed seromuscular grafts for urinary tract reconstruction.[6] A review of some of the clinical problems is presented by Cordonnier.[7]

Biochemical changes engendered by bringing urine into contact with the intestinal mucosa were, of course, available for study only after procedures such as the ureterosigmoidostomy had been performed. From the viewpoint of absorption, there would be some difference if the intestinal mucosa exposed to urine were in a discrete pocket, or still part of the main intestinal tract (in the latter case, a greater number of bacteria and a larger surface area would be present). Two considerations might indicate that difficulties would be encountered in either type of preparation.

1. The composition of urine differs markedly from the gut contents.
2. Absorptive capabilities of the bladder[8] and intestine are not alike.

Following ureterosigmoidostomy, hyperchloremic acidosis has been repeatedly observed.[9,10,11] That is, there occurs a blood picture characterized by a high chloride content and a lowered pH. The bicarbonate level is depressed; as a compensatory mechanism, $CO_2$ is expelled by the lungs (teleologically, in an effort to restore the ratio $H_2CO_3/BHCO_3$). The blood concentration of sodium may be normal, while potassium is often low; the nonprotein nitrogen tends to be elevated (Table 18 summarizes these findings).

TABLE 18

CHEMICAL FINDINGS IN A TYPICAL CASE OF HYPERCHLOREMIC ACIDOSIS ASSOCIATED WITH URETEROSIGMOIDOSTOMY

| *Blood Component* | *Milliequivalents Per Liter* | |
|---|---|---|
| Sodium | 142 | (Normal) |
| Potassium | 3 | (Low) |
| Chloride | 126 | (High) |
| Bicarbonate | 13 | (Low) |
| Non-protein nitrogen (mg%) | 62 | (High) |

Pathogenesis of the disorder may be as follows. After isotonic saline is experimentally placed in the gut lumen, the chloride content can be observed to decrease, while the bicarbonate content increases.[20] This intestinal absorption of chloride and secretion of bicarbonate lowers the blood $HCO_3^-$ level and elevates the $Cl^-$ content. When this is added to the inability of the damaged kidney to conserve bicarbonate, the stage is set for hyperchloremic acidosis. Following ureterosigmoidostomy, it has been observed that venous blood from the sigmoid colon has higher values of ammonia, chloride, and urea than peripheral blood.[21] In addition, the pH is lower (more acidotic).

Continuous absorption of chlorides and ammonia are two of the major factors in the development of this picture.[12,13,14] Renal damage,[10] and pressure differences between the urinary tract and gut contribute to the difficulty (intrarectal pressure is higher than intravesical). To further compound the difficulties encountered, hypopotassemia has been observed as a frequent component of the hyperchloremic acidotic picture. This likely arises from the inability of the damaged kidney to conserve potassium, and the passage of potassium from the gut wall to the fecal mass. The potassium level may be low enough to result in muscle weakness, frank quadriplegia and electrocardiographic evidences of hypokalemia.[11] Often there is no specific correlation between the degree of muscle impairment and the extent of potassium depression as indicated by the electrocardiogram. (See chapter on Potassium Metabolism.) Normochromic anemia and transient disturbances of carbohydrate metabolism[12] have occasionally been noted to accompany the hyperchloremic acidosis secondary to ureteroenterostomy (perhaps also due to electrolyte disturbances). In addition, with a ureterosigmoidostomy, 'secondary' changes in electrolytes may result from

(a) Constriction of the ureteral orifices,
(b) Stone formation with obstruction, and
(c) Ascending infection.

The two possibilities to account for the rise in nonprotein nitrogen (increased absorption of nitrogenous products and renal damage), have also been widely discussed as the agents responsible for elevation of the blood nonprotein nitrogen following gastrointestinal hemorrhage.[15] Since absorption is slight in the very lowest segment of the bowel, attempts have been made to implant the ureters far down into the rectum. However, as long as the rectal sphincter remains intact, urine refluxes into the large gut, and is available for absorption. Intestinal bacteria can convert a number of nitrogenous products of the urine into ammonia. This cause of $NH_3$ genesis has been implicated in some cases of ammoniagenic coma.[16] In such instances, the oral administration of nonabsorbed antibiotics to diminish the bacterial population, may be helpful.

In the treatment of hyperchloremic acidosis, accompanying ureterosigmoidostomy, the following measures have been advocated.

1. Forcing fluids.
2. Low chloride diet.

3. Frequent emptying of the rectum (to diminish the contact time between urine and gut).
4. Administration of sodium bicarbonate (to counteract the acidosis).

Control of urinary tract infection, and the institution of ureteral drainage is of great importance. If marked hypokalemia is present, potassium administration may be advisable, even if the NPN is somewhat elevated. The acidosis often results in a depression of blood ionized calcium and supplements of this electrolyte are in order. Frequent checks on the blood levels of sodium, potassium, chloride, calcium and nonprotein nitrogen, are mandatory.

In summary, urine may be diverted into isolated intestinal pouches, or directly into the alimentary tract. A picture of hyperchloremic acidosis can result, as well as hypopotassemia. The causative mechanism may be divided between intestinal uptake of chlorides and ammonia with concomitant bicarbonate loss, and renal damage. In addition, intestinal segments, including reversed seromuscular grafts, have been used for repair of the ureters.[17,18] Electrolyte disturbances may be infrequent in these latter cases, since the urine is in contact with the intestine for only a brief period of time. Sections of everted small intestine have also been used to absorb ascitic fluid,[19] but possible electrolyte disturbances due to the procedure are unknown.

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# 29

# MISCELLANEOUS DISORDERS

A NUMBER OF little understood disorders which affect the intestinal tract are discussed in this chapter. These entities are important, not because of the total number of patients afflicted, but because of the insight they give into the reactions of the intestinal tract to diverse stimuli.

## UREMIA

Sixty per cent of cases of uremia coming to autopsy show pathologic alterations in the gastrointestinal tract.[1] Edema of the mucosa and submucosa, and hemorrhage are the most frequently encountered changes. Ulcerative and necrotic lesions also occur but are less frequent. The pertinent literature on this subject, up to 1952, has been summarized.[1] These pathologic findings occur in cases of 'renal azotemia' (due to renal insufficiency) and not in instances of 'prerenal azotemia,' such as the elevation of blood nonprotein nitrogen which follows severe gastrointestinal bleeding. Uremia is associated with profound changes throughout the entire organism. The hemostatic mechanism, for instance, can be deranged, perhaps due to platelet alterations.[3] The resulting bleeding tendency is likely one of the causes for oozing of blood into the alimentary canal during uremia.

Theories to account for gastrointestinal lesions in uremia are numerous; they can be summarized as follows.

1. Urea is excreted by the gut wall, and converted to ammonia in the lumen; this latter compound is irritative to the mucosa.

2. Azotemia is associated with thrombosis of small vessels in the alimentary canal; this is the basic process, with ulcerations the result.

3. Changes in the gastrointestinal apparatus are due to an interaction of factors such as elevated blood pressure, blood vessel damage, and nervous stimuli due to irritation.

4. Urea is itself the irritating factor. Evidence has been presented both for and against this hypothesis. The weight of facts at present, however, indicate that urea per se is not the cause of the alterations. During a uremic episode, many substances fail to be excreted and are concentrated within the body. The intestinal tract does not escape this accumulation of materials, and it has been shown, for example, that potassium increases in the gastrointestinal mucosa during uremia.[4] Passage of urea through the alimentary mucosa into the lumen[5,15] exposes the compound to bacterial action. The enzyme urease, possessed by multiple bacterial species, splits urea to ammonia and carbon dioxide. That this is truly a bacterial function is shown by complete inhibition of the process in animals treated with antibiotics.[6] Ammonia produced this way is usually small; it is absorbed and enters the portal circuit.

Therapeutically, a few points follow from this discussion.

1. If ammonia generation is a factor in the gastrointestinal lesions of uremia, then antibiotic administration to 'sterilize' the gut and diminish bacteria which split urea to ammonia is justified.

2. Again assuming ammonia to play a role in the alimentary lesions seen in uremia, the use of compounds which bind ammonia (resins), stimulate the urea cycle in the liver, or otherwise remove ammonia, would appear to be worthy of trial.

3. Blood within the alimentary lumen is broken down to a number of nitrogenous products which, upon absorption, raise the blood level of nonprotein nitrogen. To prevent this added bleeding insult during uremia, platelet administration is used when there is a thrombopenia.

4. Removal of the products of uremia may be attempted, by means of artificial dialysis. If such a device is not available, peritoneal lavage[7] is capable of removing quantities of nitrogenous and other 'wastes.' Lavage of the alimentary lumen can also be rewarded by the removal of electrolytes and nitrogenous products.[8] So effective is the procedure, that it may be used to tide a patient over acute uremia. The use of a three lumen tube for this purpose

has been reported.[9] With the tip of the tube low in the small intestine, lavage was carried out. It proved possible to control alterations in serum electrolytes induced by the procedure, by changing the composition of the lavage fluid and administering electrolytes intravenously. Dialysis through loops of exposed gut has also been reported to be effective.[2,14]

5. Massive gastrointestinal bleeding in a patient with uremia should not immediately lead to the conclusion that a uremic lesion is responsible. Cases of uremia have been reported in which massive alimentary hemorrhage has been due to other causes.[10]

Uremia, or even milder renal impairment, alters a number of studies used in evaluating the gastrointestinal tract. When cobalt$^{60}$-vitamin $B_{12}$ is given by mouth, even though pernicious anemia is not present, reduced urinary excretion occurs in patients with renal disease.[11] Such individuals usually absorb the vitamin well, in distinction to persons with pernicious anemia, but take longer to excrete a dose via the diseased renal apparatus. Uremia may also result in an elevation of the blood amylase value,[12] making differential diagnosis difficult. However, the blood amylase concentration is little affected during water diuresis in normal subjects.[13]

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## TUMORS OF THE INTESTINAL TRACT

The functionally important segment of the digestive tract between the pylorus and the ileocecal valve, that is the entire small intestine, is not a frequent site of tumor formation. The greatest majority of small intestinal tumors are benign,[1] and some run a course of many years duration.[2] The infrequent malignant tumors display a variety of degrees of invasiveness,[3] and can have bizarre manifestations (for example, lymphosarcoma may interfere with lymphatic drainage, with resulting malabsorption from the small gut[4]).

Tumors of the large intestine are more frequent in occurrence, and there are even reports of bizarre metastatic lesions to the large bowel.[5] Information as to the genesis of intestinal tumors is far from complete, but certain factors have been implicated.

1. Intestinal polyps occur in approximately five per cent of the adult population. Occasionally, polyps removed while the subject is still asymptomatic show malignant characteristics; whether this represents malignant change in a benign lesion, or whether the polyp was always malignant is not certain.[9]

2. Multiple polyposis of the colon is a recognized genetically determined disorder (q.v.). The polyps may oft times be found to be malignant. About one third of the cases are sporadic, and likely represent new mutations; the gene is perhaps dominant with re-

duced penetrance. The concept has arisen that such intestinal polyps are but one manifestation of a more generalized disorder termed 'familial adenomatosis.'[6]

3. The hereditary entity of familial small intestinal polyposis with melanin pigmentation of the buccal mucosa (Peutz-Jeghers syndrome), is rarely accompanied by malignant degeneration of the polyps.[7] There are probably a number of variants of this condition, some of which are accompanied by more widespread alimentary polyposis.[8]

4. Chronic ulcerative colitis is associated with the formation of 'pseudo-polyps' as surrounding tissue is sloughed. These polyps sometimes show malignant changes.[9] This is one instance in which young individuals may develop gastrointestinal malignancies.[19]

5. Occasionally tumors which have cutaneous manifestations will produce lesions in the gastrointestinal tract. Among these are familial multiple telangectasias, and Kaposi's hemorrhagic sarcoma (both of which may result in gastrointestinal bleeding).

Experimentally, there are few methods available for producing intestinal malignancies.

1. Severance of the autonomic recurrent nerve in the insect Leucophea results in the development of tumors of the anterior portion of the gastrointestinal tract.[10]

2. The subcutaneous injection of bile and derivatives in Syrian hamsters has been reported to be followed by gastrointestinal neoplasms and adenomatous polyps.[11]

3. The administration of hydrocarbons in food,[32] or in drinking water, results in production of intestinal adenocarcinomas in strain A mice.[12] Interestingly 2-naphthylamine which is carcinogenic to the urinary bladder has little effect on loops of the sigmoid colon used as bladder substitutes.[24]

4. Irradiation of the glandular stomach in mice is followed by tumor formation.[26]

5. There is a report that introduction of heterologous deoxyribonucleic acid in the mouse was accompanied by intestinal carcinoma.[25]

6. By capsule implantation, intestinal tumors can be made to grow in animal hosts.[33]

7. Croton oil, a cocarcinogen, has been shown to damage the absorptive powers of the pig intestine;[34] whether prolonged administration would lead to neoplastic changes has not been determined.

Interrelations between intestinal tumors and surrounding structures are relatively unknown. Rats bearing a carcinosarcoma lose weight from the mesentery at a greater rate than the general body weight.[13] The mesentery, however, serves as a depot area for lipid,[28] and the weight loss may represent nothing more than the effects of inanition. An unusual case of epiploic lipomatosis has been described, in which the epiploic tags of the large bowel were tremendously hypertrophied;[14] whether this represents a benign tumor is unknown. Multiple primary and metastatic tumors can invade the retroperitoneal area, mesentery, and peritoneum.[15]

There are few studies on the biochemical changes in gastrointestinal tumors.[29] A comparison of the enzymatic activity of transplanted adenocarcinomas of stomach and intestine in mice, with their normal counterparts, has been reported.[16] The tumors investigated were developed by the administration of hydrocarbon emulsions in drinking water.[12,17] Pepsin and rennin were present in the normal gastric mucosa, but vanished in the neoplastic tissue. An enzyme depolymerizing thymonucleic acid, however, was present to the same extent in normal and pathologic tissues.

The greatest activity of alkaline phosphatase, per gram of tissue, is found in the small intestine.[18] The enzyme nearly disappears in gastric and intestinal adenocarcinomas. Acid phosphatase is reduced, in gastric and intestinal adenocarcinomas, to half the value in the normal mucosa.[18] Gomori[20] states that acid phosphatase can be found in practically all cells of the gastrointestinal epithelium, except parietal cells (which possess an adenosinetriphosphatase). He further claims that carcinomas of the stomach may display a high concentration of the enzyme, which can also be found in the gastric juice. Burt and coworkers [35] discussed phosphatases in induced gastrointestinal tumors and noted that their levels were depressed.

Fishman[21] found $\beta$-glucuronidase activity to be elevated in 37 out of 44 gastrointestinal neoplasms; a correlation with blood levels of the enzyme was not recorded. Gomori[20] reported that phosphamidase occurred in large amounts in malignant epithelial tumors, and that polyps of the intestine gave a uniformly positive reaction, despite their histologic appearance (suggesting their possible malignant potential).

Laboratory adjuncts in the diagnosis of intestinal tumors are unfortunately not always of immediate aid. Available studies include the following.

1. Demonstration of blood in the stool.
2. Intubation, and possible peritoneoscopy.
3. Roentgen studies (with care to avoid administering radio-opaque insoluble preparations by mouth, if an obstructing lesion is present at a lower level).
4. Exfoliative cytology may be of value in identifying abnormal cells.[22,27] The use of fluorescent dyes to show the presence of nucleic acids in malignant growths, which has been employed in the detection of gynecological malignancies,[23] has potential usefulness in classifying cells recovered from the gastrointestinal tract.
5. Carcinoid tumors liberate serotonin, which is excreted by the urinary system. Examination of urine for 5-HIAA levels can thus be of diagnostic aid (see chapter on Carcinoid Syndrome).
6. There are three entities which may simulate intestinal cancer by the production of tumors and a distorted cellular pattern—these are the lesions of mucormycosis,[31] the ameboma[30] and lymphopathia venereum. Stool studies, a Frei test, and biopsy of any accessible lesion are all essential in preventing diagnostic errors.

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## GASTROINTESTINAL AMYLOIDOSIS

Amyloid derived its name from the fact it was originally considered 'starch-like.' Present views are that the substance is protein in character, and more than a single entity (since staining differences occur [1]). The great affinity of amyloid for the dye congo red has been made the basis of a test for amyloid deposits. Congo red is administered intravenously, and the blood stream sampled after a few minutes. In the presence of significant amyloid, over 90 per cent of the dye will have been removed from the circulation.

The mechanism of amyloid deposition is unknown. There is evidence that amyloid is produced in mice exposed to daily gamma irradiation,[2] or Freund's adjuvants,[6] but the clinical correlation in man is not clear. A 'primary' type of amyloidosis is rarely encountered in which there occurs involvement of muscle bundles in the tongue and heart. By far the commonest types of amyloidosis are 'secondary' to another disorder (most often chronic infection, multiple myeloma, or Hodgkin's disease). Secondary amyloidosis affects the gastrointestinal tract in 40 per cent of its victims.[3] The alimentary canal is the fourth most commonly attacked site, following the spleen, kidney, and adrenals.[4] Stomach, small intestine, colon, and esophagus are involved in that order of frequency.[3] The roentgenographic appearance of gastrointestinal amyloidosis has been summarized.[3,5] There is widening and thickening of intestinal folds at necropsy, with areas of hemorrhage. The statement has been made that the defect of absorption observed in gastrointestinal amyloidosis is proportional to the degree of mechanical block that is produced.[3] Amyloidosis of the digestive canal has been reported to mimic ulcerative colitis.[4] Rarely, disorders of the intestine (ulcerative colitis and regional enteritis) may be associated with amyloid deposits elsewhere in the body.

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## PARASITES

The intestinal canal and its appendages are subject to attack by a wide variety of parasites.[1] Biochemical reasons for each species of parasite having its own region of preference would be a worthwhile contribution. The great majority of ingested parasites stay within the gut lumen, from which point they may produce roentgenologic evidence of their presence.[2] The principal diagnostic aid, however, is the finding of a parasite or its ova in the stool; in rare cases, a parasite may be ingested and passed without infestation becoming established.

Certain parasites pass from the intestine into the body proper. The best known examples are Trichinella spiralis and Endamoeba histolytica (whose penetrating ability is likely related to enzymes which damage the gut wall). Such infestations give rise to esoinophilia and occasionally to the production of antibodies. Schistosomes enter the body via the skin and then migrate, through the blood stream, to the wall of the alimentary canal. Infestation by these organisms can often be diagnosed by the use of rectal biopsy,[3,4] which reveals the parasite in the gut mucosa.

Hookworms lodged in the intestine produce bleeding into the lumen. By the use of blood tagged with radiochromium ($Cr^{51}$), Roche and coworkers[5] estimated the blood loss per hookworm (Necator americanus) to be $3 \times 10^{-2}$ ml per day. The worm Ancylostoma duadenale produced even a greater blood loss. In terms of the number of ova passed, the average daily loss of blood was 2.74 ml of blood per 1000 ova per gram of stool. Because of this continuing slow oozing of blood, hookworm infestation has been proposed in the treatment of polycythemia vera.[15]

One of the most intriguing effects of parasites on the alimentary canal is destruction of the intramural nervous plexus in the esophagus by the neurotoxin set free by dead parasites of Chagas disease (Trypanosoma cruzi). Köberle states that this toxic effect is the most frequent cause of megaesophagus in Brazil.[6] Another little understood effect is the action of intestinal parasites on enzyme activity within the lumen. Ascaris lumbricoides, for instance, produces an inhibitor of trypsin.[7]

Infestation with the fish tape worm, Diphyllobothrium latum, is rarely (0.1 to 0.2 per cent of all cases) associated with a clinical picture approximating pernicious anemia.[8] The gastric mucous membrane in this disease resembles that found in true pernicious anemia, and achlorhydria can be present.[9] Absorption of radioactive vitamin $B_{12}$ is low in cases of 'fish tape worm pernicious anemia'[10] and rises following expulsion of the worm.[11] For success in establishing the anemia, Ernberg and Brante[8] concluded that the worm was favored by a high site of implantation in the intestine (where it could reach incoming vitamin $B_{12}$), that the host was disfavored by low intrinsic factor production and by a poor supply of vitamin $B_{12}$. The role played by intrinsic factor in the genesis of tape worm pernicious anemia is not agreed upon. It has been reported that administration of intrinsic factor improves the blood picture.[12] Other investigators, however, conclude that lack of vitamin $B_{12}$ is the major pathogenic factor, since the worm actively takes up the vitamin.[13]

Parasites have been mentioned in the development of tumors in the rat forestomach.[14] In man, E. histolytica occasionally results in a tumor referred to as an ameboma. The specific stimuli for these exuberant growths in response to parasites is not known.

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## 'COLLAGEN' DISEASES

Rarely is the intestine the primary site of disorders involving connective tissue.[1] During the course of a disease such as scleroderma, gastrointestinal symptoms transiently occur; these can make definitive differentiation between primary alimentary pathology and secondary reactions to the main disorder quite difficult.[2]

Scleroderma can involve the gut,[3] although more often distinctive changes are observed in the esophagus.[4] Periarteritis nodosa can also be accompanied by alimentary symptoms such as nausea and vomiting. Hemorrhage from a gastric vessel during the course of periarteritis nodosa may lead to hematemesis or melena, while that from an intestinal vessel can cause bloody diarrhea.[5] Retroperitoneal hemorrhage sometimes occurs in the course of the disease and is followed by a picture resembling an abdominal emergency. Experimentally, periarteritis-like changes have been produced in the gut of rats by the administration of desoxycorticosterone acetate and salt.[9] Small arteries and arterioles are also involved in the gut during lupus erythematosus, and an acute surgical abdomen may be simulated.[6]

Structure of the connective tissue of the gut has been little studied. The gastrointestinal tract possesses mucoitin sulfuric acid, which contains D-glucosamine, in contrast to the more common chondroitin sulfuric acid (built around galactosamine) of other tissues.[10] The stomach contains soluble precursors of collagen, whereas the gut does not.[7] Spencer has reported that the integrity of intercellular binding of mucosal cells of the intestine, in vitro, is disturbed by proteinases and hyaluronidase, but not by agents which split disulfide bonds.[8]

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## 'NONSPECIFIC' ULCERS OF THE INTESTINE

The gastrointestinal tract is beset by a number of ulcerations which differ in etiology and location. The most frequently occurring type is peptic ulceration of the stomach or duodenum of 'idiopathic' origin. Drug-induced peptic ulcers also occur, and Kirsner has provided a review of the subject.[1] Isolated ulcers of the alimentary canal occasionally appear during the course of regional enteritis, ulcerative colitis, uremia, parasitic or bacterial infections, and following the sloughing of a polyp.

A limited number of patients, however, have one or more ulcers of the small intestine that appear to be 'primary' (and not the result of any of the above defined causes [2]) . Zollinger and Ellison [3] related some of these cases to a common factor; they noted instances of jejunal ulceration in association with islet cell tumors of the pancreas. The tumors did not produce insulin—hence hypoglycemia, a potent stimulus of gastric acid, was not a contributory factor. The occurrence of peptic ulceration in patients with multiple endocrine tumors has been noted as a separate entity.[4] The complex interrelationship of the pancreas and the intestine has also been emphasized by a report of Verner and Morrison.[5] These authors described a syndrome of watery diarrhea and low blood potassium in two patients suffering from pancreatic islet tumors. The relationship of this disorder to that described by Zollinger and Ellison is uncertain.

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